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Limitations and opportunities for immune checkpoint inhibitors in pediatric malignancies

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Abstract

Immune checkpoint inhibitors (ICI) have shown great promise in a wide spectrum of adult solid and hematological malignancies, achieving objective tumor responses and prolonging survival. However, there is limited clinical success amongst pediatric patients. In this review, we summarize the current understanding of ICI and present an up-to-date overview of recent and ongoing clinical trials of ICI in pediatric malignancies. In addition, we will discuss immunologic and clinical difficulties in this young population, as well as future prospects for combination of ICI with other immune-based and conventional treatments.

Keywords

Immune checkpoint inhibitor; Immunotherapy; Programmed death receptor-1 (PD-1); Programmed death-ligand 1 (PD-L1); Cytotoxic T lymphocyte antigen-4 (CTLA-4)

Introduction

Remarkable advances in cancer immunotherapy in recent years have led to paradigm shifts in oncology. The most noticeable results have been with T-cell-based therapies including immune checkpoint inhibitors (ICI), genetically engineered T-cells and bispecific antibodies (BsAb). T-cells represent a major class of cellular drugs in immunosurveillance and tumor eradication with exquisite specificity and long-term memory. However, during tumor equilibrium or progression, T-cells become exhausted or tolerized to tumor cells [1,2]. A cardinal feature of T-cell exhaustion is the overexpression of inhibitory receptors, including programmed death receptor-1 (PD-1, CD279), cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152), lymphocyte-activation gene-3 (LAG-3), T-cell immunoglobulin domain and mucin domain-3 (TIM-3), IL-10 receptor, killer immunoglobulin receptors, among others. Some of these checkpoint molecules exert their immunosuppressive effects by down-regulating the normal T-cell response and increasing $FoxP3^+$ regulatory T-cells (Tregs) numbers and activity [3,4]. Monoclonal antibody (mAb) based therapies to counteract these checkpoint molecules can remove the brake that restrains tumor-infiltrating T-cells, thereby achieving

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significant clinical benefits in different malignancies including advanced melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC) [5–8]. However, most of the studies to date are focused on adult cancers and little is known about the role of these ICI in pediatric malignancies. Here, we review the clinical use of these ICI and their limitations regarding toxicity and efficacy in the context of pediatric malignancy. Furthermore, we will discuss the potential for combining ICI with other proven and investigational therapies in children.

Immune checkpoint inhibitors and clinical trials

CTLA-4 antibodies

CTLA-4, type I transmembrane glycoprotein that belongs to Ig superfamily, is constitutively expressed on memory T-cells and Tregs, which is critical in preventing self-reactive T-cells from inducing autoimmunity [9]. It is homologous to CD28 and shares the same B7 ligands, B7-1 (CD80) and B7-2 (CD86), but it has a negative effect on T-cell activation. Several suppressive mechanisms for T-cell functions have been attributed to CTLA-4 (Fig. 1). Ipilimumab (IgG4 isotype) was the first CTLA-4 inhibitor to demonstrate overall survival benefit in metastatic melanoma [6,10]. Another CTLA-4 inhibitor, tremelimumab (IgG2 isotype), has also been proven successful in metastatic melanoma and other malignancies [11]. Although the pediatric experience is very limited, a substantial number of clinical trials have extended the age eligibility to patients <=18 years of age (Table 1). In the first report of ipilimumab for advanced solid tumors in pediatric patients, although no major response was noted, some tumor regression was noted to be durable [12]. A comparison of ipilimumab to high-dose interferon (IFN) α-2b among pediatric patients with high-risk melanoma is ongoing, and the combination with IL-2, vaccine, or CD19-chimeric antigen receptor (CAR) expressing T-cell therapy are being tested in patients with metastatic melanoma and advanced malignancies.

PD-1/PD-L1 antibodies

PD-1 is expressed on T-cells following T-cell receptor (TCR) engagement and it declines after resolution of acute inflammation. However, under chronic antigen exposure, PD-1 remains high on chronically activated T-cells that become exhausted [13,14]. Several Inhibitory mechanisms for T-cell functions have been ascribed to PD-1 (Fig. 2). Two PD-1 ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), can engage PD-1 to render T-cell dysfunctional, and to maintain the exhausted T-cell phenotype [15]. While PD-L2 is exclusively expressed on activated dendritic cells and macrophages, PD-L1 has a broad tissue distribution including tumor cells and is rapidly induced by inflammatory mediators (e.g. IFN-γ, lipopolysaccharides, GM-CSF, IL-4 and IL-10). PD-L1 is expressed in many pediatric cancers including leukemia (42–100%), lymphomas (27–80%), glioma (75–100%), Wilms tumor (14%), soft tissue sarcomas (STS) (58%), and metastatic osteosarcoma (75%) [16,17], and upregulation of PD-L1 was consistently associated with poor clinical outcomes [17–25].

PD-1/PD-L1 inhibitors enhance anti-tumor immune response by restoring T-cell cytotoxic function, resulting in anti-tumor effect, while facilitating the generation of memory T-cells

to provide long term anti-tumor response [26–28]. Nivolumab, a fully human IgG4 mAb, induced objective response in melanoma, NSCLC, and RCC patients (18% to 28%), and most responses were durable for more than 1 year [8,16]. Pembrolizumab, another humanized monoclonal IgG4 anti-PD-1 antibody, also showed high clinical response rates that were durable (37% with median of 11 months) in patients with advanced melanoma [29]. In addition, pidilizumab (CT-011), a humanized IgG1 monoclonal anti-PD-1 antibody, has mainly been investigated in advanced hematologic malignancies [30–32]. Other anti-PD-L1 antibodies (e.g. atezolizumab (MPDL32890A), durvalumab (MEDI4736), and avelumab (MSB0010718C)), inhibit binding of PD-L1 to PD-1 and CD80, without blocking the interaction between PD-L2 and PD-1. They have demonstrated clinical activity with acceptable toxicities, not only against immunogenic cancers, such as melanoma and RCC, but also against less immunogenic epithelial cancers such as NSCLC, colorectal, gastric, and cervical and bladder cancers [33–35]. Most notable was the substantially milder toxicity profile of PD-1/PD-L1 blockades, when compared to anti-CTLA-4-mediated immune related adverse events (irAEs) [36–38].

In contrast to the enthusiasm for the PD-1/PD-L1 inhibitors in adult cancers, few studies have been carried out in pediatric cancers. Blumenthal et al. reported their experience of pembrolizumab in patients with recurrent brain tumors including 5 pediatric patients, but they failed to show a benefit on overall survival [39]. The obvious exceptions are pediatric patients with refractory Hodgkin lymphoma (HL) which have shown durable responses to pembrolizumab [40,41]. Recently, pembrolizumab was approved by FDA for HL in adult and pediatric patients, and this is a first approval of PD-1 inhibitor for pediatric use. Children's Oncology Group (COG) is conducting a phase I/II study of nivolumab alone or in combination with ipilimumab for relapsed or refractory solid tumors (NCT02304458) and phase I/II study of pembrolizumab for advanced melanoma or PD-L1 positive advanced solid tumors or lymphoma (NCT02332668).

Anti-LAG-3 antibody

Lymphocyte-activation gene-3 (LAG-3, CD223) is another vital immune checkpoint that may have a synergistic interaction with PD-1/PD-L1 [42,43]. LAG-3 is a member of the Ig superfamily and exerts a wide variety of biologic impacts on T-cell function via binding to major histocompatibility complex (MHC) class II with high affinity [44]. It is expressed on activated T-cells, Tregs, NK cells, B-cells, tumor-infiltrating lymphocytes (TILs), and dendritic cells [45–49]. The LAG-3/MHC class II molecule interaction inhibits CD4+ T-cell proliferation and cytokine secretion. Co-expression of LAG-3 and PD-1 correlates with greater T-cell exhaustion, accompanied by impairment of CD8+ effector T-cell function, best demonstrated in chronic viral infection [48,50–52]. They synergistically regulate T-cell function, blunt anti-tumor immune response, and promote tumoral immune escape [43,52].

Although anti-LAG-3 antibody (Immutep, IMP321) failed to demonstrate objective responses, this agent was well tolerated and appeared to correlate with development of CD8+ T-cell effectors [53–55]. When combined with paclitaxel, outcome in breast cancer was improved [56,57]. Although there has been no open clinical trials for pediatric patients so far, and most clinical trials have not yet extended the age eligibility to patients <=18 years

of age, clinical trials combining with PD-1 inhibitors are actively recruiting patients with advanced solid tumors (NCT0196819) and recurrent glioblastoma (NCT02658981) to assess the safety, tolerability and efficacy.

Anti-B7-H3 antibody

Human B7-H3 (CD276) is another member of the B7/CD28 Ig superfamily with activating as well as inhibitory roles that regulate T-cell function [2,58,59]. While the receptor for B7-H3 has yet to be discovered, structural studies in the mouse suggested a receptor engagement on T-cell involving the particular segment (FG loop) of the B7-H3 [60,61]. B7-H3 preferentially down-regulates type I helper T-cells (TH1)-mediated immune response and inhibits T-cell proliferation and cytokine production [62–64]. B7-H3 protein showed broad mRNA expression on many tissues and cell types with proven functions on cellular responses, including proliferation, apoptosis, adhesion, and metastasis [65–67]. While its protein expression is restricted in normal tissues, much higher levels are found in human malignancies [68–75], and because of its association with highly aggressive tumor behavior and poor clinical outcome, B7-H3 has utility as a tumor-associated antigen as well [71,74,76].

Anti-B7-H3 antibodies block the inhibitory effects of B7-H3 on T-cells and enhance the efficacy of autologous T-cells against tumors [77,78]. Anti-B7-H3 murine mAb 8H9 has shown promise as a radioimmunoconjugate in xenograft models, and clinically, intrathecal or intra-Ommaya^{131}I-8H9 has demonstrated efficacy in controlling CNS metastasis of neuroblastoma [79]. Currently, intraperitoneal ^{131}I-8H9 is also being tested among adolescent and young adults with desmoplastic small round cells tumor (DSRT), while intra-pontine ^{124}I-8H9 using convention enhanced delivery is applied to diffuse intrinsic pontine glioma (DIPG), with minimal toxicity and encouraging results (Table 1). The humanized and affinity matured forms of 8H9 and its epitope dependence on the integrity of the FG loop were recently reported [75]. Another humanized mAb, enoblituzumab (MGA271) specific for B7-H3, has shown anti-tumor activity in preclinical models of RCC and bladder cancer [77]. Clinical trials of MGA271 as a single agent for refractory cancers that express B7-H3 in pediatric patients (NCT02982941) and advanced prostate cancer in adults (NCT02381314), as well as in combination with ipilimumab (NCT02381314) or pembrolizumab for refractory cancer (NCT02475213) are in progress. Although clinical responses to the standard anti-B7-H3 IgG alone have not been encouraging, its combination with other ICI or even with conventional therapies could offer opportunities. Novel engineered forms such as B7-H3 × CD3 bispecific antibodies may be valuable alternatives [80]. When the receptor(s) and signaling pathways of B7-H3 become better defined, more effective anti-B7-H3 inhibitors could potentially be explored.

Limitations of immune checkpoint inhibitors

Safety in children

Despite the overall success of ICI in adults, safety data in children have been hard to come by. In adults, ipilimumab-related adverse events were often mild to moderate, but occurred in more than 70% of patients, and these toxicities correlated with its dosage [6,81]. Meta-

analysis of 18 clinical trials showed that CTLA-4 inhibitors at higher doses (10 mg/kg) were associated with a higher risk of treatment-related mortality (TRM) [82]. Enterocolitis, hepatitis, and dermatitis were most commonly observed, and these irAEs were associated with tumor responses and favorable outcome [12,81,83–85]. Approximately 30–50% of patients experienced adverse skin reactions including rash and itching, one third enterocolitis, 2–9% hepatotoxicity (some life-threatening), 1.5% hypophysitis whose symptoms included fatigue, headaches, myalgia, loss of appetite, nausea and vomiting [6,81]. Hypophysitis with adrenal insufficiency is potentially fatal and requires urgent attention and treatment [86]. Neurologic toxicities were rare, but life-threatening neuropathies (e.g. Guillan-Barré syndrome, severe motor neuropathy, myasthenia gravis, aseptic meningitis) and optic neuritis have been reported, which required immediate stoppage of the ICI [85,87]. Most irAEs could be controlled with high-dose corticosteroids which did not seem to impair the antitumor effects of ipilimumab [88]. In children the incidence of grade 3 or 4 $irAE_S$ was 27%, and the spectrum included pancreatitis, pneumonitis, colitis, and hepatitis, similar to those among adult studies [12].

The toxicity profile of PD-1/PD-L1 blockades was less severe than that of CTLA-4, with no significant increase in TRM in a meta-analysis [82], with an overall incidence of 7–14% grade 3 and 4 toxicities [8,89,90]. Most notably, PD-1/PD-L1 inhibitors showed a slightly different toxicity profile including organ-specific inflammatory conditions such as pneumonitis rather than colitis [91]. The most common adverse events were fatigue, with an incidence of 16%–37% with anti-PD-1 and 12–24% with anti-PD-L1, followed by fever, chills, and infusion reactions [91]. Dermatologic toxicities such as rash, pruritis, and vitiligo were frequently observed, and vitiligo was more common than with ipilimumab (10% vs. 2%, respectively) [92]. Colitis, endocrine toxicities, and hepatic toxicities have been described, but most were less extensive than anti-CTLA-4 mAb [91,92]. While pneumonitis was rarely reported in the studies of anti-CTLA-4 mAb alone, up to 10% of patients receiving anti-PD-1/PD-L1 therapy developed this complication, leading to 3 TRM in the early phase of nivolumab [8]. In addition, among patients including children treated with anti-PD-1 mAbs, critical neurologic and endocrinologic adverse reactions have been reported, and some of them being irreversible [82,93–95]. These unpredictable off-target toxicities to critical organs, besides being life-threatening, are particularly concerning for children in whom the organs are less mature and potentially prone to life-long disabilities [91].

Efficacy in children

Another uncertainty in pediatric application of ICI is their uncertain benefit and the lack of appropriate predictive biomarkers. Although clinical trials have shown tumor responses in some patients, few are major responses and most have not been durable [12,39–41]. There is a need to better understand the mechanisms of action of PD-1/PD-L1 in these pediatric tumors, to determine if there is an underlying genetic resistance to ICIs [13,96]. While PD-L1 expression in adult tumors has been proposed as a potential biomarker for response [8,37], PD-L1 expression is often heterogeneous within tumors, and can be even discordant between primary tumor and metastases [17,97]. Furthermore, PD-L1 negative tumors also

responded to PD-1 blockade or combination treatment with nivolumab and ipilimumab [38,98,99].

Recent whole genome and exome sequencing of tumors have identified the role of neoantigens and the importance of pre-existing T-cell clones and the mutational threshold for adequate response to ICI. High frequencies of nonsynonymous mutational burden and tumor neoantigens, as well as mutations in DNA repair pathways were strongly associated with therapeutic benefit following anti-CTLA-4 and anti-PD-1 antibodies [96,100]. Neoantigen-reactive CD8-T-cells are responsible for tumor regression after PD1 blockade, suggesting that ICI enhances neoantigen-specific T-cell reactivity [96]. However, given the heterogeneity of most human tumors, neoantigens, if not essential for tumorigenicity, could be downregulated or even lost, leading to tumor escape. An analysis of 27 cancer types showed that the median frequency of non-synonymous mutations varied by more than 1,000-fold across the cancer types [101]. While melanoma and lung cancer exceeded 100/Mb, most pediatric cancers showed frequencies as low as 0.1/Mb (one mutation per exome). If each mutation creates a neoantigen capable to stimulate a T-cell clone, more mutations and more neoantigens should produce a more robust T-cell response. ICI are now known to be more effective in highly mutated cancers, with a mutational threshold estimated at 100 mutations per exome (3.3 mutations/Mb) [100,102], an unfavorable prerequisite for most pediatric solid tumors. The highest mutation frequencies are attributable to extensive exposure to carcinogens, such as UV light in melanoma and tobacco smoking in lung cancers [101], not generally associated with pediatric cancers. Although pediatric tumors express PD-L1, ICI alone may have less or even no effect in pediatric cancers when compared to melanoma and NSCLC [103,104]. However, somatic mutation frequencies can vary widely across patients within a cancer type and even across sites within the same patient [101,105,106]. As present, the role of ICI is being tested for hypermutated malignancies in children with biallelic mismatch repair deficiency (NCT02992964).

Another consideration is the tumor microenvironment as a major component of resistance to immunotherapy. In this context, adult cancers are thought to be preceded by a long-term chronic inflammatory phase such as infections (hepatitis B or C, human papillomavirus, Epstein-Barr virus (EBV), Helicobacter pylori) or repeated exposures to irritants or carcinogens [107]. In contrast, the preneoplastic period in pediatric cancer is much shorter, often only weeks or months, and except in the case of EBV-induced Burkitt lymphoma, an association between preceding inflammation and typical pediatric cancers (i.e., small round blue cell tumors) is not clear. Whereas TILs, a heterogeneous population of lymphocytes growing within a tumor [108], in adult cancers are peritumoral, forming focal inflammatory cell aggregates of diverse cell types, including T-cells and NK cells among macrophages and dendritic cells (DC), TILs in pediatric tumors are scarce and scattered among macrophages. Moreover, these macrophages are commonly CD163/CD68+, the phenotype of M2 tumor-associated macrophage (TAM), which often comprise 60% to 70% of the cellular infiltrate [109]. While M1 TAMs are highly inflammatory and effective killers for microorganisms and tumor cells, M2 TAMs are generally anti-inflammatory, by secreting IL-10 and TGF-β while failing to secrete other proinflammatory cytokines, as well as immunosuppressive, protecting tumor cells from NK cells and T-cells during tumor progression [110–114].

TILs, particularly those anti-tumor type I T-cells, were associated with better patient survival and seemed to play a critical role in response to ICIs in many adult cancers [115–117]. A general consensus is the absence of clinical response if TILs are absent or insufficient [118]. Clinical scientists have coined the descriptor ‘hot’ for tumors that have high numbers of TILs (T-cell-inflamed) e.g. melanoma, and the opposite end of the spectrum as ‘cold’ (not T-cell-inflamed) e.g. prostate cancer and most pediatric cancers (Fig. 3) [119]. The potential mechanisms for immune evasion in the T-cell-inflamed or ‘hot’ tumors include inhibition by upregulation of ICI (PD-1/PD-L1 and CTLA-4), expression of indoleamine-2,3-dioxygenase (IDO), recruitment of Tregs, loss of antigen expression, and T-cell intrinsic anergy. Increased IDO expression by APCs induces tryptophan depletion, resulting in antigen-specific T-cell anergy, and Tregs recruitment and activation, resulting in T-cell dysfunction. On the other hand, in non-T-cell-inflamed or ‘cold’ tumors, immune escape mechanisms include a lack of innate immune recognition, infiltration by M2 TAMs, paucity of dendritic cell infiltration, lack of chemokines for effective T-cell trafficking, dense sessile stroma with high density of fibroblasts, and a hostile extracellular matrix restricting T-cell access. In addition, altered oncogene pathways could also cause immune escape, e.g. p53 mutation results in decrease of innate immune activation and a lack of T-cell infiltration, inactivation of phosphatase and tension homolog (PTEN) can enhance tumor cell survival and proliferation through increased AKT activity, and activated signal transducer and activator of transcription 3 (STAT3) signaling reduces recruitment of both DC and T-cells and plays inhibitory roles in anti-tumor immunity [119–122]. While hot tumors have a chance to benefit from ICI, cold tumors may require additional strategies to promote T-cell homing into and function within these tumors [121].

Other relevant considerations regarding anti-tumor T-cell immunity in children include: (1) most pediatric solid tumors exhibit low or absence of MHC, which presents antigens on tumor cells and could be critical for both the afferent and the efferent arm of the T-cell response, potentially compromising the prospects of an effective T-cell immunity to any neoantigen, (2) immature immune system in young children, (3) dose-intensive chemotherapy in children and hence profound lymphopenia and immunosuppression, (4) immature or altered gut microbiome that could also affect the response to ICI [123–128]. The potential impact of gut microbiome on tumor microenvironment and effectiveness of chemotherapy viewed in the context of drastic changes of gut flora with age and antibiotics is just emerging [129–131]. For these reasons, ICI alone is likely to be insufficient to control pediatric tumors. However, as BsAb or CAR T-cells can retarget polyclonal T-cells to tumor, their combination with ICI may offer novel potentials for some pediatric malignancies (See below) [132,133].

Future directions

The clinical success of ICI in adults has paved the way for evidence-based combinations with conventional therapeutics or additional ICI, with the goal of both additive and synergistic effects to improve the survival of patients.

Combination with other ICI

The combination of ipilimumab and nivolumab was initially studied in advanced melanoma and demonstrated a 40% objective response rate, with 30% of patients exhibiting >80% tumor reduction [134]. Phase II/III trials of this combination also showed impressive response rates (61%) and significantly improved outcomes in melanoma, and even in PD-L1-negative tumors [98,99]. This combination has also been studied in metastatic RCC with acceptable safety and high response rates of 39% (NCT01472081) and in recurrent glioblastoma after standard therapy with surgery, RT, and temozolomide in a phase III trial (NCT02017717). Phase I/II clinical trial for recurrent or refractory solid tumors in children are also recruiting patients (NCT02304458). Similar combinations, pembrolizumab plus ipilimumab, or durvalumab plus tremelimumab are ongoing in prior-treated NSCLC, advanced melanoma and refractory or advanced stage rare tumors. Several other combinations using ICI targeting LAG-3, B7-H3, and TIM3 have shown encouraging results in the pre-clinical models and are currently in clinical trials.

Combination with chemotherapy

Recent studies have also shown that combinations of various chemotherapies with ICI can have synergistic effects. The combination of ipilimumab and dacarbazine has improved the overall survival of patients with metastatic melanoma when compared to dacarbazine monotherapy [10]. Nivolumab in combination with platinum-based chemotherapy for advanced NSCLC also showed an encouraging outcome with a 2-year OS rate of 62%. Although irAEs were greater than those expected with nivolumab monotherapy, most toxicity was manageable [135]. A number of phase I studies are currently underway in NSCLC and other solid tumors including pediatric cancers, aimed at investigating the safety and tolerability of combining ICI with standard chemotherapies [135,136].

Combination with targeted agents

Another strategy is to combine with targeted agents including tyrosine kinase inhibitors (TKI), BRAF inhibitors and anti-angiogenic agents. Despite high response rates with these targeted therapies, most patients progress within 1 year because of acquired resistance through a number of mechanisms, including immune escape via the PD-1/PD-L1 and other immune checkpoint pathways [137,138]. The effective treatment of human gastrointestinal stromal tumors (GIST) with imatinib is associated with an increased intratumoral CD8+effector T-cells (CD8)/Tregs ratio [139]. Increased Tregs could compromise the immune response to tumors, as shown by imatinib-resistant tumors, where the lower CD8/Treg ratios were correlated with increases in immune checkpoint molecules [139–141]. A phase I study of ipilimumab plus imatinib in advanced solid tumors (NCT01738139) and a phase I/II clinical trial of ipilimumab with dasatinib in GIST or STS are ongoing (NCT01643278). The combination of the anti-PD-L1 mAb, durvalumab, and the epidermal growth factor receptor (EGFR)-TKI gefitinib in NSCLC have shown promising clinical activities with mild treatment-related AEs [142].

BRAF inhibitors which target driver mutations in the tumor cells can promote adaptive immunity, but, can concurrently upregulate T-cell exhaustion markers including PD-1 and TIM-3 and PD-L1 on tumor cells, consistent with a potential immune resistance [143].

Although a phase I study of vemurafenib with ipilimumab was terminated prematurely due to hepatotoxicity [144], $BRAF^{V600}$ inhibitors plus ICI should produce synergy given the high response rates from BRAF inhibitors and the durable remissions induced by ICI [145,146]. Currently, there are several open or pending clinical trials in lymphoma, advanced melanoma, RCC and other refractory solid tumors studying ICI in combination with targeted agents (NCT02465060, NCT02224781, NCT02027961 and NCT02858921).

Anti-angiogenic mAbs against vascular endothelial growth factor (VEGF), such as bevacizumab, and multi-targeted TKIs, such as sunitinib and pazopanib, have also been tested in combination with anti-PD-1/PD-L1 mAbs. Blockade of VEGF produced immunomodulatory effects, which included promoting dendritic cell maturation and effector T-cell trafficking, while decreasing myeloid-derived suppressor cells (MDSCs), Tregs and suppressive cytokines at the tumor microenvironment [147–149]. Combination of bevacizumab and ipilimumab has been studied in glioblastoma and advanced melanoma, showing promising activity with manageable toxicity profile [150,151]. Bevacizumab with anti-PD-L1 inhibitor, atezolizumab, also showed clinical activity without exacerbation of irAEs, and phase III clinical trial of this combination is ongoing in advanced RCC (NCT02420821).

Combination with radiotherapy (RT)

Tumor irradiation has immunologic effects, such as increased tumor antigen presentation, increased chemokine release, and recruitment of effector T-cells to the tumor microenvironment, although potentially deleterious effects can also be induced, such as upregulation of PD-L1, secretion of TGF-β, and induction of Tregs [152–155]. Localized RT has an abscopal effect on nonirradiated tumor sites through immunostimulation, which could be exploited and combined with immunotherapy [156–158]. While radiation shapes the TCR repertoire of the expanded peripheral clones, anti-CTLA-4 mAb promotes expansion of T-cells and contraction of Tregs; hence, their combination may have synergistic benefit [159–162]. Studies in prostate cancer and melanoma combining RT with ipilimumab showed clinical antitumor activity and manageable irAEs [158,163]. Although another study in advanced melanoma failed to demonstrate significant benefits of anti-CTLA-4 inhibitor, it did show persistent T-cell exhaustion in melanoma with high PD-L1 could be reversed by PD-L1 blockade. The authors suggested that the combination of radiation, anti-CTLA-4 and anti-PD-L1 mAbs might promote more potent anti-tumor immune response [164]. Clinical studies to determine the safety and efficacy of RT with various ICI are currently underway to identify the optimal radiation dose, radiation fractionation, and dose and timing of ICI.

Combination with T-cell based therapies

Adoptive T-cell therapy using CAR T-cells or BsAb (blinatumomab) specific for CD19 has been major breakthroughs in the treatment of acute lymphoblastic leukemia (ALL) [165,166]. When non-clonal T-cells are gene-modified with CAR or armed with bispecific antibodies [132,167], they mediate potent anti-tumor cytotoxicity, leading to strong T-cell activation and production of proinflammatory cytokines. However, despite promising clinical responses (e.g. CD19-directed T-cell based immunotherapy), tumor recurrence was observed, partly because of genomic instability and the effects of cancer immune editing

[168]. Additional resistance mechanisms include downregulation or loss of target antigen expression, tumor-associated dendritic cell dysfunction, increased Tregs, immunosuppressive cytokines, activation of alternative signaling pathways, and anti-antibody formation [66,93,168–170].

T-cells driven by CAR or BsAb can trigger tumor cells to develop various immunosuppressive strategies, resulting in the release of inhibitory factors and a hostile tumor microenvironment, leading to T-cell exhaustion and tumor escape [168]. Upregulation of checkpoint molecules has been suggested as one of the main mechanisms of adaptive resistance in adoptive T-cell therapies [171], and evidence has continued to accumulate to support a key role of the PD-1/PD-L1 axis in attenuating anti-tumor immune responses [172,173]. Although PD-1/PD-L1 expression may not be robust at the time of diagnosis, they can be rapidly induced following blinatumomab treatment and is associated with disease relapse and resistance [174,175]. Cytokine-release syndrome (CRS), one of the major side effects of both CAR T-cells and BsAbs, results from massive cytokine secretion (IFN-γ, IL-6 and IL-10) associated with T-cell engagement and proliferation [176], leading to upregulation of PD-1/PD-L1 expression and immune resistance [174,177]. Blockade of PD-1/PD-L1 signaling could significantly increase anti-tumor cytotoxicity and T-cell proliferation and activity [171]. Given the significant acute (CRS) and chronic (B cell aplasia) toxicities from CD19-directed immune therapies, addition of ICI could intensify these side effects.

Combination of blinatumomab and pembrolizumab was administered in a pediatric patient with ALL. She was refractory to blinatumomab, and her blasts showed high PD-L1 expression. She was treated with blinatumomab and pembrolizumab after transplant and attained a remission without significant toxicities or exacerbation of CRS [174]. A phase I study of blinatumomab in combination with nivolumab or both nivolumab and ipilimumab in patients with relapsed or refractory CD19+ precursor B-acute leukemia has started (NCT02879695). Combined treatment with BsAbs [178–180] and various ICI including anti-PD-L1, anti-CTLA-4, anti-LAG-3, and anti-B7-H3 could be alternative therapeutic strategies in refractory or relapsed cancers in children, although toxicities could become prohibitive.

CAR T-cell therapy is another highly promising immunotherapy for children and adults with B-cell leukemia. However, the clinical results in solid tumors have not been encouraging. For optimal tumor eradication, CAR T-cells must have proper target antigen selection, co-stimulatory signaling, and the ability to move into the tumor, as well as persistence or proliferation, while avoiding T-cell exhaustion and T-cell death, now believed to be a major limiting factor [181]. Recent clinical trials have shown that tumor burden and chemotherapy conditioning before CAR T-cell therapy are critical, and it is likely that CAR T-cell therapy alone will be insufficient for cure [182]. Combination with ICI could be a future direction. Blocking PD-1/PD-L1 can unleash the cytotoxic functions of adoptively transferred T-cells, and potentially promote the development of endogenous T-cells that target neoantigens [168,183].

The antitumor effect of combinational therapy with CAR T-cells and PD-1 inhibitor was investigated preclinically using transgenic Her2 mice treated with Her2-specific CAR T-cells. Tumor Her2 antigen triggered PD-1 upregulation in CAR T-cells, and PD-1 blockade enhanced Her2-specific T-cell functions and decreased MDSC in the tumor microenvironment, leading to enhanced anti-tumor effect [36]. A clinical trial to study the combination of CD19-CAR T-cells and ipilimumab in patients with B-cell lymphoid malignancies including pediatric patients has been initiated (NCT00586391).

Conclusion

Although overall survival rates for pediatric malignancies approached upwards of 80% [184], further improvements have slowed down in the past decades, despite the use of dose-intensive genotoxic therapies approaching their toxicity limits. New approaches in pediatric patients with advanced stage, relapsed or refractory cancers are desperately needed. Several novel therapeutic agents including small molecular targeted agents, monoclonal antibodies, and T-cell based immunotherapies have shown promise. To fully exploit these powerful modalities against tumors with low mutational load and weak TIL content (‘cold’ tumors), emphasis should be placed on proven and novel strategies to drive T-cells selectively and quantitatively into cold tumors, to activate them to proliferate inside a hostile tumor microenvironment, and to avoid exhaustion or activation induced cell death. Once T-cells can infiltrate, persist, proliferate and survive, the addition of ICI should vastly enhance their potential in any of these pediatric malignancies. However, there remain significant hurdles with regard to both acute and late toxicities. A concerted effort should be made not to run redundant studies, but to systematically confront these limitations for a more streamlined pediatric integration. Combination treatment should be the framework and no single approach, whether cell therapy or antibody will likely be curative.

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Highlights

1. Immune checkpoint inhibitors (ICI) have achieved a great success in adult cancers.
2. There is growing interest in ICI for pediatric malignancies.
3. There are significant barriers for successful pediatric integration of ICI.
4. Combination with other treatment modalities could be a promising option.

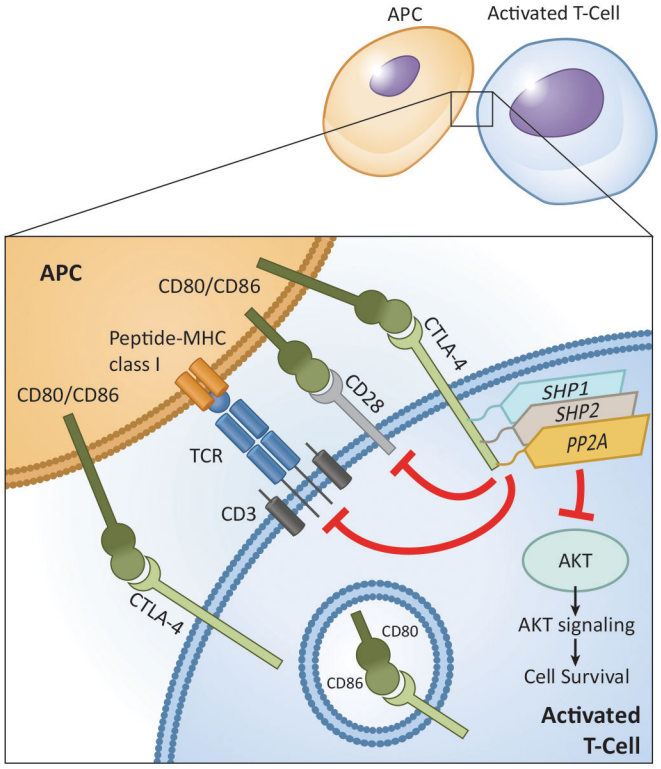

Fig. 1.

Resting T-cells rarely express CTLA-4, which is retained inside the secretory granules, but after TCR activation, CTLA-4 is up-regulated and emerges to the plasma membrane of T-cells and binds to B7 ligands (CD80 and CD86) on antigen presenting cells (APCs) with 10–100-fold higher avidity than CD28, resulting in reduced T-cell proliferation and lessened cytokine secretion [185,186]. CTLA-4 exerts TCR inhibitory signal through serine/threonine protein phosphatase 2 (PP2A) and Src-homology 2 domain-containing phosphatase 2 (SHP2), and induces inhibition of serine/threonine kinase AKT on the downstream of phosphatidylinositol-3-kinase (PI3K), resulting altered T-cell metabolism and decreased T-cell proliferation and activity [187,188]. Besides, CTLA-4 shortens the duration of immune synapses as a result of signal attenuation and integrin deactivation and increases the T-cell activation threshold by producing inhibitory signals in the early phase of tumorigenesis.

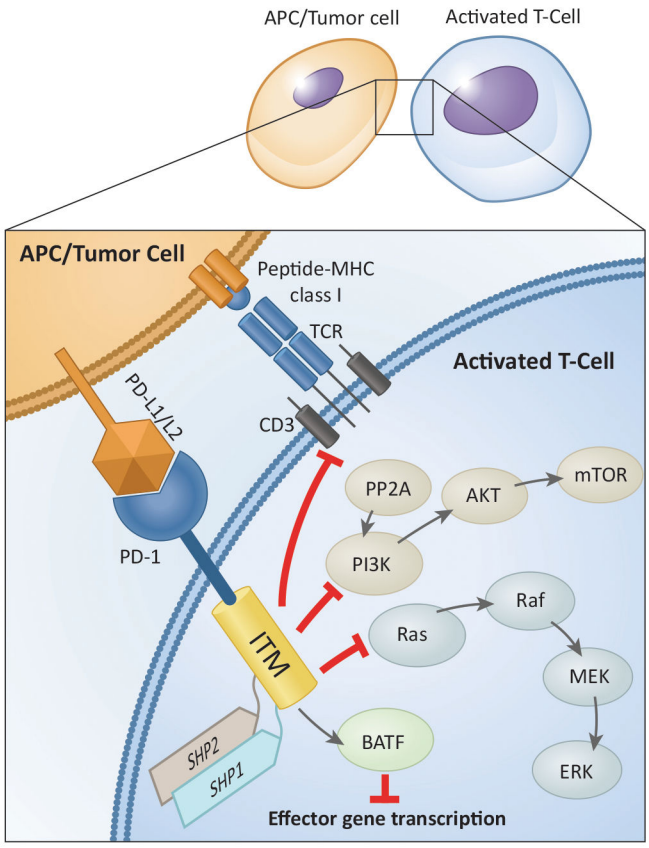

Fig. 2.

PD-1 is also expressed on T-cells following TCR engagement and activation. PD-1 and PD-L1 ligation exerts inhibitory signals for lymphocyte activation. PD-1 modulates T-cell function through (a) direct antagonism of TCR signaling by recruiting Src-homology 2 domain-containing phosphatase (SHP)-1 and SHP-2 to tyrosine-based inhibitory motifs (ITM; immunoreceptor tyrosine-based motifs) in the PD-1 tail, (b) inhibition of PI3K/AKT/mechanistic target of rapamycin (mTOR) pathway, implicating the role of PD-1 in metabolism, nutrient sensing, survival, and cell growth to cell cycle, (c) modulation of Ras pathway, linking PD-1 to cell cycle and reducing T-cell proliferation, (d) increased expression of basic leucin zipper transcription factor, activating transcription factor (ATF)-like transcription factor (BATF), which can repress expression of effector gene transcription [14,189,190]. Further, these signaling events impair T-cell motility and stability leading to unproductive immune synapses with APCs [170,191].

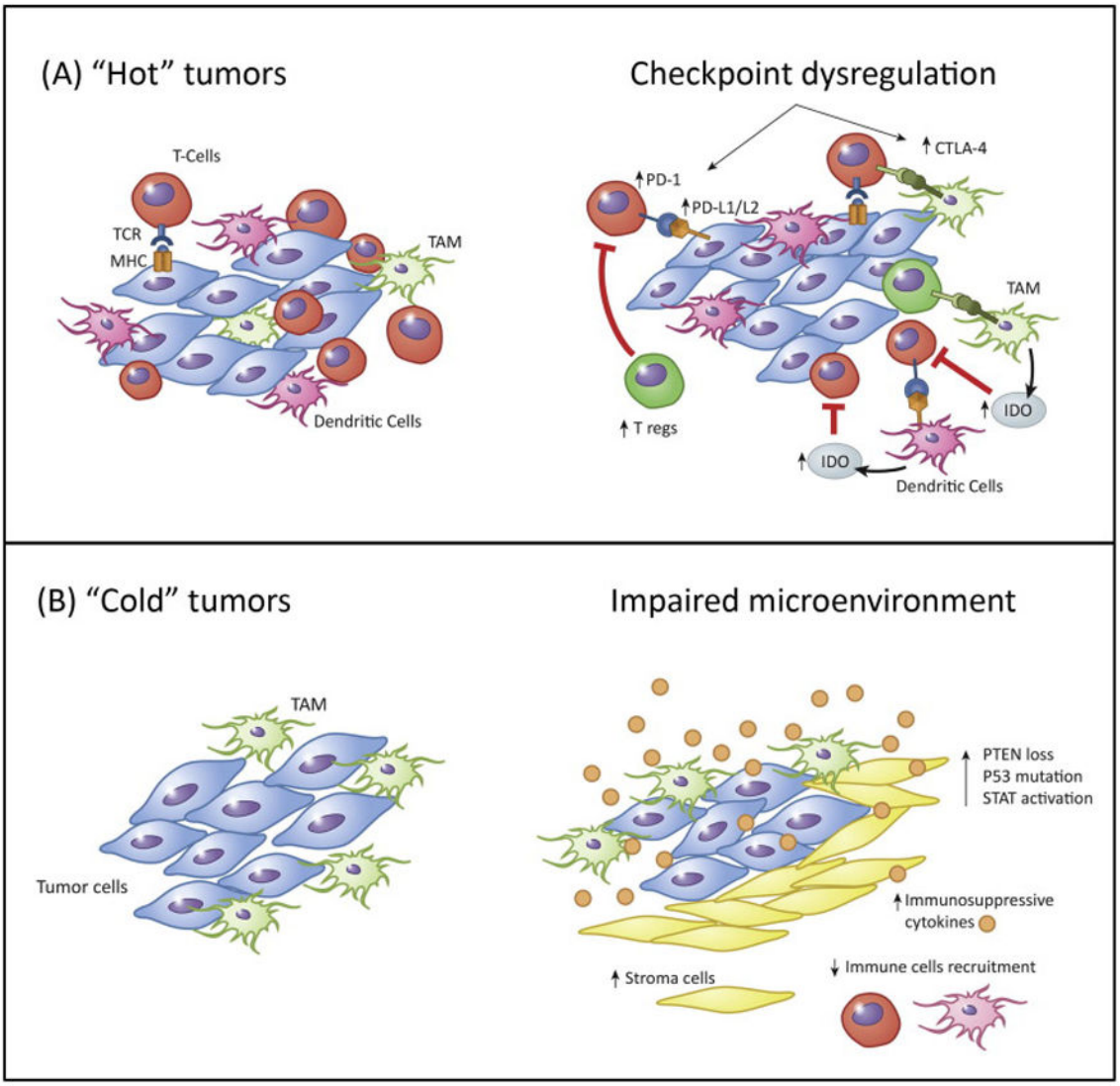

Fig. 3.

Mechanisms of tumor immune escape. 'Hot' tumors (A) may escape through up-regulation of immune checkpoint molecules and Tregs, secretion of immunosuppressive factors, indoleamine-2,3-dioxygenase (IDO), or T-cell anergy. (B) Tumor intrinsic mechanism of escape in "cold tumors" by downregulation of MHC molecules, attraction of M2 tumor-associated macrophages (TAMs), alteration of the tumor microenvironment, discouraging T-cell homing either by subduing inflammation, or suppressing release of T-cell chemokines, or releasing inhibitory cytokines to impair the recruitment of immune cells to the tumor microenvironment, or by dysregulating oncogene pathways including PI3 kinase/PTEN/AKT, p53 and STAT3 signaling.

Table 1

Clinical Trials of immune checkpoint inhibitors for which pediatric patients <=18 are eligible

Target	Agent	Indication	Age (years)	Phase	Clinical trial (NCT)	Results
CTLA-4	Ipilimumab	Stage III or IV melanoma	12–17	2	NCT01696045	Terminated due to slow accrual
	Ipilimumab	Treatment resistant cancer (sarcoma, Wilms tumor, lymphoma, neuroblastoma)	3–21	1	NCT01445379	Completed
	Ipilimumab	Advanced synovial sarcoma	≥13	2	NCT00140855	Terminated due to poor accrual
	ipilimumab	Metastatic renal cell carcinoma	≥ 16	2	NCT00057889	Completed
	Ipilimumab plus imatinib	Advanced cancers	≥15	1	NCT01738139	Recruiting
	Ipilimumab plus paclitaxel	Metastatic melanoma	12–70	2	NCT01827111	Active, not recruiting
	Ipilimumab or interferon α-2b	Resected stage III or IV melanoma	>12	3	NCT01274338	Active, not recruiting
	Ipilimumab plus peginterferon α-2b	Stage III or IV melanoma	≥16	1	NCT01496807	Active, not recruiting
	Ipilimumab plus IL-2	Metastatic melanoma	≥16	1,2	NCT00058279	Completed
	Ipilimumab plus CD19-CAR T cell	B cell NHL, ALL, CLL	All ages	1	NCT00586391	Active, not recruiting
	Ipilimumab plus gp100 peptide vaccine	Stage IV melanoma	≥ 16	2	NCT00032045	Completed
	Ipilimumab plus gene modified T cells and dendritic cell vaccine	Locally advanced or metastatic malignancies	≥ 16	1	NCT02070406	Recruiting
	Ipilimumab ± gp100 peptide and montanide ISA-51 vaccine	Stage IV melanoma	≥ 16	2	NCT00077532	Completed
PD-1	Nivolumab	Refractory or recurrent hypernutated malignancies in biallelic mismatch repair deficiency positive patients	1–18	1,2	NCT02992964	Active, not recruiting
	Nivolumab	Glioblastoma	≥ 1	2	NCT02550249	Recruiting
	Nivolumab plus cyclophosphamide	Relapsed pediatric solid tumors	1–21	1,2	NCT02901145	Not yet recruiting
	Nivolumab plus anti-GD2 antibody (Ch14.18/CHO)	Relapsed or refractory NBL	1–18	1	NCT02914405	Not yet recruiting
	Nivolumab plus brentuximab vedotin	Hodgkin lymphoma after failure of 1st line therapy	5–30	2	NCT02927769	Recruiting
	Nivolumab plus brentuximab vedotin	Relapsed/refractory NHL	≥15	1,2	NCT02581631	Recruiting
	Nivolumab plus EBV specific T cells	Relapsed/refractory EBV positive lymphoma	All ages	1	NCT02973113	Recruiting
	Nivolumab ± stereotactic radiosurgery	Recurrent, advanced, or metastatic chordoma	≥15	1	NCT02989636	Not yet recruiting
	Nivolumab plus ICE chemotherapy (Ifosfamide, carboplatin, and etoposide)	Relapsed/refractory Hodgkin lymphoma	≥15	2	NCT03016871	Not yet recruiting
	Nivolumab plus gene-modified T cells, and NY-ESO-1 vaccine therapy	Stage IV or locally advanced solid tumors expressing NY-ESO-1	≥16	1	NCT02775292	Not yet recruiting

Target	Agent	Indication	Age (years)	Phase	Clinical trial (NCT)	Results
	Nivolumab plus cyclophosphamide ± radiotherapy	Relapsed/refractory malignancies	≤ 18	1,2	NCT02813135	Recruiting
	Pembrolizumab	Recurrent, progressive, or refractory high-grade gliomas, diffuse intrinsic pontine glioma, or hypermutated tumors	1–29		NCT02359565	Recruiting
	Pembrolizumab	Advanced melanoma or advanced, relapsed/ refractory PD-L1-positive solid tumors or lymphoma	0.5–17		NCT02332668	Recruiting
	Pembrolizumab	Advanced bone and soft tissue sarcoma	≥ 12	2	NCT02301039	Active, not recruiting
	Pembrolizumab plus IL-2	Stage III–IV melanoma	≥15	2	NCT02748564	Not yet recruiting
	Pembrolizumab plus cyclophosphamide, fludarabine, IL-2, and tumor infiltrating lymphocyte infusion	Metastatic melanoma	16–70	2	NCT02621021	Recruiting
	Pembrolizumab plus axitinib (anti-VEGF receptor antibody)	Alveolar soft part sarcomas and other soft tissue sarcomas	≥16	2	NCT02636725	Recruiting
	Pembrolizumab plus 3rd generation GD-2 CAR T cell	Relapsed/refractory NBL	All age	1	NCT01822652	Active, not recruiting
	Pembrolizumab plus GSK3359609 (anti-inducible T cell co-stimulator (ICOS) receptor agonist antibody)	Advanced solid tumors	≤ 18	1	NCT02723955	Recruiting
PD-L1	Atezolizumab	Solid tumors which failed to primary treatment	≤ 30	1,2	NCT02541604	Recruiting
	Avelumab	Recurrent or progressive osteosarcoma	12–49	2	NCT03006848	Recruiting
	Durvalumab	Relapsed or refractory solid tumors, lymphoma, and CNS tumors	1–17	1	NCT02793466	Recruiting
PD-1 and CTLA-4	Nivolumab± Ipilimumab	Relapsed/refractory solid tumors or sarcoma (metastatic or unresectable solid tumors)	1–30	1,2	NCT02304458	Recruiting
	Nivolumab plus Ipilimumab	Untreated, unresected or metastatic melanoma	≥15	3	NCT02905266	Recruiting
		Resected stage III and IV melanoma	≥16	2	NCT02970981	Not yet recruiting
	Nivolumab plus blinatumomab ± ipilimumab	Poor risk relapsed/refractory CD19+precursor B-lymphoblastic leukemia	≥16	1	NCT02879695	Not yet recruiting
	Nivolumab plus NY-ESO-1 vaccine ± ipilimumab	Resected stage IIIC/IV melanoma	≥16	1	NCT01176474	Active, not recruiting
PD-L1 and CTLA-4	Durvalumab plus tremelimumab	Advanced rare tumors	≥16	2	NCT02879162	Recruiting
B7-H3	Enoblituzumab (MGA271)	B7-H3 expressing relapsed or refractory solid tumors	1–30	1	NCT02982941	Recruiting
	^{131}I-8H9	Desmoplastic small round cell tumors and other solid tumors involving the peritoneum	≥1	1	NCT01099644	Recruiting

Target	Agent	Indication	Age (years)	Phase	Clinical trial (NCT)	Results
	^{131}I-8H9	Relapsed/refractory or advanced CNS or leptomeningeal cancer	All ages	1	NCT00089245	Recruiting
	^{124}I-8H9	Non-progressive diffuse pontine glioma previously treated with external beam radiation	3–21	1	NCT01502917	Recruiting
IDO	Indoximod + temozolomide	Progressive primary malignant brain tumors	3–21	1	NCT02502708	Recruiting