Targeted immunotherapy for pediatric solid tumors

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ABSTRACT
Metastatic and refractory pediatric solid tumor malignancies continue to have a poor outcome despite the > 80% cure rates appreciated in many pediatric cancers. Targeted immunotherapy is impacting treatment and survival in these aggressive tumors. We review current promising immunotherapeutic approaches in the pediatric oncology solid tumor setting.

Abbreviations: ADCC, antibody dependent cellular cytotoxicity; ALK, The anaplastic lymphoma kinase; CARs, Chimeric Antigen Receptors; COG, Children’s Oncology Group; CTLs, cytotoxic T-lymphocytes; DC, dendritic cells; GD2, Disialoganglioside; GMCSF, granulocyte colony macrophage stimulating factor; GPNMB, Glycoprotein non-metastatic B; IGF-1R, Insulin-like growth factor-1 receptor; IL-2, interleukin 2; mAb, monoclonal antibody; PD1, Programmed Death Receptor 1; RMS, rhabdomyosarcoma; TRAIL, Tumor necrosis factor related apoptosis-inducing ligand.

KEYWORDS
Immunotherapy; pediatric oncology; solid tumors

Introduction
Pediatric solid tumors include those located within the central nervous system (CNS), and those outside the CNS, non-neural tumors. Despite the successes realized in the treatment of many pediatric malignancies, control of these metastatic or recurrent solid tumors remains a challenge with less than 20% survival.1 For those who survive, late sequelae of chemotherapy and radiation therapy is the rule.2 Novel treatments including immunotherapy are desperately needed that can impact survival while decreasing late effects. Although the number of solid tumor immunotherapy clinical trials in pediatric patients has been small compared to adults, the results are promising even in patients with advanced disease and must be explored further. Herein we review the current immunotherapeutic approaches for the most common non-neural pediatric solid tumors including: bone and soft tissue sarcomas, neuroblastoma, Wilms tumor, and hepatoblastoma.

Monoclonal antibodies (mAbs)

Cell surface immune targets

Gangliosides
Disialoganglioside (GD2) is a carbohydrate antigen expressed normally on the tissues of the CNS, peripheral nerves and skin melanocytes whose true function is not known. GD2 is expressed uniformly on neuroblastoma, melanoma, and osteosarcoma while some sarcomas, lung tumors and brain tumors may also express GD2.3,4 Murine, mouse-human chimeric and humanized versions of anti-GD2 antibodies have been studied in neuroblastoma clinical trials.5 The first generation of anti-GD2 mAbs included 3F8, 14G2a, and ch14.18. Reversible pain, fever, tachycardia and urticaria were the most common toxicities reported in initial trials of murine 3F8 and14G2a antibodies. Phase I and II studies revealed moderate responses in patients with relapsed/recurrent neuroblastoma. In a later phase II study 3F8 was combined with granulocyte colony macrophage stimulating factor (GM-CSF) to augment granulocyte/monocyte antibody dependent cellular cytotoxicity (ADCC). Patients tolerated this combination without significant toxicity and those with bone marrow involvement benefited most.4 14G2a antibody, was utilized in a phase I study combined with interleukin 2 (IL-2) to augment NK mediated ADCC and responses were also noted. The mAb ch14.18 is a chimeric mouse-human antibody with a longer half-life than 14G2a, which also showed efficacy in an initial pilot study and a phase II trial in children with recurrent/refractory neuroblastoma.6,7 Both studies included IL-2 and GM-CSF to enhance ADCC.6,7 This led to a Children’s Oncology Group (COG) phase III study in newly diagnosed high-risk neuroblastoma. In this trial patients were randomized to receive immunotherapy with ch14.18 antibody combined with alternating IL-2 and GM-CSF. Patients randomized to receive immunotherapy had significantly improved rates of event-free survival (66±5% vs. 46±5% at 2 y, p = 0.01) and overall survival compared to standard therapy alone.7 These results were so compelling that the COG Neuroblastoma Committee decided to offer ch14.18 to prior trial participants who had been randomly assigned to the no immunotherapy arm.8 Subsequently ch14.18, dinutuximab, was FDA approved as part of first line therapy for high-risk neuroblastoma patients in early 2015.

GD2 has recently been found to be uniformly present in osteosarcoma human cell lines with several having a higher average expression than neuroblastoma cell lines.5,9
Osteosarcoma patients with recurrent disease whose cell lines were tested were found to maintain expression of GD2 making this a meaningful target. An upcoming COG Phase II study will therefore evaluate dinutuximab in relapsed/refractory osteosarcoma patients (personal communication Gorlick).

Second generation GD2 mAbs have been developed and are in early clinical trials. HuL4.18-II.2 is a fusion protein of a humanized second-generation anti-GD2 antibody and II.2. Phase I and II studies of patients with refractory/relapsed neuroblastoma have been completed and toxicities were similar to ch14.18. The phase II study revealed a response rate around 21% for patients with non-bulky disease. Another second-generation mAb (Hu14.18K332A) is undergoing clinical study in patients with recurrent/refractory neuroblastoma, melanoma, osteosarcoma and Ewing’s sarcoma (NCT00743496). This is a humanized mAb with a mutation to alanine at lysine 322 in order to limit complement fixing and thus pain associated with the anti-GD2.

Unlike GD2, GD3 is a GD that is not expressed on normal tissues. GD3 expression is found in melanomas, soft tissue sarcomas, and tumors of neuroectodermal origin. Similar to GD3, N-glycolated ganglioside NeuGc-GM3, GM3, is expressed primarily in neoplasms and not in normal tissues. GM3 has been used targeted in breast cancer and will likely be used to target neuroblastoma, nephroblastoma and Ewing’s sarcoma in the future.

**B7-H3**

The surface immunomodulatory glycoprotein B7 homolog three protein (B7-H3) is overexpressed in some human tumors and inhibits natural killer cells and T cells leading to promotion of tumor growth in multiple cancers including neuroblastoma. The murine antibody 8H9 is specific for the B7-H3 protein, 4Ig-B7H3 and has been radiolabeled with 131I to target solid tumors in xenografts. 131I-8H9 has been utilized in a Phase I study along with radiolabeled anti-GD2 antibody 3F8 to target patients with a CNS metastases of neuroblastoma. Either 131I-8H9 or 131I-3F8 was given intrathecally to 21 patients (1 patient received both mAbs, 17 patients 131I-8H9, and 3 patients 131I-3F8) along with systemic chemotherapy of irinotecan and temozolomide. There was minimal toxicity and 17 patients remained free of CNS neuroblastoma with a median of 33 mo.

**RANK-L**

The cytokine RANK-L is a TNF family member expressed on the surface of osteoblasts and is released by activated T-cells. RANK-L has been found to be critical to osteoclast formation, function and survival. Dysregulation in bone remodeling has been found to be key in the pathophysiology of bone metastasis and RANK-L plays an essential role in this process. Denosumab is a humanized mAb that binds RANK-L ligand and has been used in phase II and III clinical trials in multiple myeloma (MM), metastatic breast and prostate cancer. Denosumab is thought to have utility in osteosarcoma because of its direct effects on bone tumor pathophysiology and expression in 75% of osteosarcomas correlating with poor response to neoadjuvant chemotherapy. Denosumab will be utilized in an upcoming COG phase II clinical trial (NCT02470091) in patients with recurrent/refractory osteosarcoma.

**Glembatumumab vedotin**

Glycoprotein non-metastatic B (GPNMB) is a type I transmembrane glycoprotein normally expressed in a variety of cell types. While its exact function is not fully elucidated, it is thought to play a role in tissue repair, cellular adhesion, and regulation of cell growth and differentiation. Aberrant or overexpression of GPNMB has been demonstrated in a variety of cancers including melanoma, breast cancer, glioma, hepatocellular carcinoma, and osteosarcoma. Glembatumumab vedotin consists of a fully-human IgG2 monoclonal antibody (CR011) conjugated to the potent microtubule inhibitor, monomethyl auristatin E (MMAE) and is thought to exert its antitumor activity by selectively delivering the potent cytotoxin MMAE to GPNMB-expressing tumor cells. It has shown activity in both metastatic/refractory breast cancer and melanoma patients in phase I and II clinical trials. GPMB gene expression was studied in osteosarcoma xenograft models and human osteosarcoma cell lines. Complete tumor responses were seen in 3/6 of the xenografts. Sixty-seven human osteosarcoma samples were tested and 92.5% expressed GPNMB. Glembatumumab vedotin induced cytotoxic effects in 74% of osteosarcoma cell lines (Roth, M. Targeting Glycoprotein NMB with Antibody-Drug Conjugate, Glembatumumab Vedotin, for the Treatment of Osteosarcoma, Pediatric Blood Cancer, accepted). Glembatumumab vedotin will be evaluated in an upcoming COG phase II clinical trial in patients with recurrent/refractory osteosarcoma (personal communication Gorlick).

**Growth factor receptors and oncogenes**

**HER2/NEU**

Her2/Neu is the epidermal growth factor receptor two oncogene that has been found to be amplified in pediatric medulloblastoma, nephroblastoma and osteosarcoma. However, its use in pediatric solid tumors has been limited. The Her2/Neu targeting mAb, Trastuzumab, was found to be safe when combined with chemotherapy in a phase II trial of newly diagnosed patients with metastatic osteosarcoma. Forty-one patients with Her2/Neu positive tumors received trastuzumab, yet survival was not significantly prolonged. As this study did not discriminate based on Her2/Neu positivity, future directions would include using trastuzumab in patients with only Her2/Neu positive tumors. Her2/Neu in pediatric tumors, similar to GD2, has recently been incorporated into chimeric antigen receptor (CAR) T cells as discussed further under CAR T cell adoptive cell transfer.

**Fibroblast growth factor receptor 4**

Fibroblast growth factors and their receptors are an integral part of normal cell development, important in regulating cell proliferations, survival, migration and differentiation. However, deregulation of these growth factors is found in many cancers and they may act as oncogenes, promoting cancer progression. There are currently MM trials utilizing antibodies to fibroblast growth factor receptor 3. Recently
fibroblastic growth factor receptor 4 (FGFR 4) was found to be overexpressed in pediatric alveolar rhabdomyosarcoma (RMS) with little expression in normal myocytes.28 RMS tumors that were highly expressing FGFR4 were associated with advanced stage and poor survival.29 Ponatinib (AP23534), a tyrosine kinase inhibitor of FGFR4, was found to be a potent inhibitor of growth in RMS cell lines and in a RMS mouse model and therefore deserves further exploration as a target in RMS.30

**Insulin-like growth factor-1 receptors**

Insulin-like growth factor-1 receptor (IGF-1R) has been found to be important in the growth of solid tumors, specifically sarcomas. In the past it was difficult to target IGF-1R because of its similarity to the insulin receptor leading to toxicities occurring without specific inhibition. Recently there has been development of humanized mAbs that target IGR-1R without major toxicities.31 The Pediatric Preclinical Testing Program evaluated the human antibody, SCH 717454 in solid tumor xenograft models and found broad antitumor activity in Ewing’s sarcoma, osteosarcoma, RMS and neuroblastoma.32 Cixutumumab (IMC-A12), a fully human IgG1 mAb against IGF-1R was used in a phase I/II trial in pediatric patients with refractory solid tumors. The drug was well tolerated, however there was limited single agent activity.33

It is thought that IGF-1R mAbs will work best in combination with other targeting agents such as mTOR inhibitors which have been shown to increase the IGF-1R serine/threonine kinase AKT. The combination of mTOR inhibition and IGF-1R AKT inhibition leads to more effective killing of RMS, osteosarcoma and Ewing’s sarcoma cell lines.31,34,35 In a recent clinical trial which included 20 patients with refractory Ewing’s sarcoma and desmoplastic small-round cell tumor, cixutumumab was combined with the mTOR inhibitor temsirolimus. This combination was well tolerated with one complete response and five partial responses.36 A COG randomized phase II study opened in December 2014 comparing standard multi-agent chemotherapy with or without the IGF-1R antibody, ganitumab in patients with newly diagnosed metastatic Ewing’s Sarcoma (NCT02306161).

**Anaplastic lymphoma kinase**

The anaplastic lymphoma kinase (ALK) gene is a receptor kinase in the insulin receptor superfamily and is expressed during neuronal development, then downregulated after birth. ALK’s expression is thought to increase tumor growth and is found in a variety of tumors including lung cancer, anaplastic large cell lymphoma, neuroblastoma, neuroectodermal tumors, glioblastoma, RMS and melanoma.12 ALK is expressed in 8–10% of neuroblastomas and germline ALK mutations are found in the majority of familial cases.6,12,37 Crizotinib was the first FDA approved ALK inhibitor and has been used successfully in early non-small cell lung cancer patients.6,38 A COG phase I study was completed in refractory solid tumors and anaplastic large cell lymphoma (NCT00939770), it was well tolerated and antitumor activity was appreciated in tumors with ALK translocations.30 Crizotinib continues to be tested in the phase II setting in relapsed/refractory solid tumors with ALK mutations (NCT00939770 and NCT02034981). A second generation ALK inhibitor, Ceritinib (LDK-378) was developed after resistance ensued in patients with primary ALK mutated lung tumors with Crizotinib.31 Ceritinib is currently being evaluated in the phase I setting in pediatric tumors with ALK mutations (NCT01742286).

**Immunostimulatory cytokines**

**TRAIL-R**

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily that has the ability to activate death receptors inducing tumor cells to undergo apoptosis. Many tumor cells express the TRAIL receptors, TRAIL-R1, and TRAIL-R2, which initiate apoptosis via TRAIL. This mechanism seems to be selective for tumors.42 It has been found that RMS, osteosarcoma and Ewing’s sarcoma cell lines which express TRAIL death receptors are sensitive to TRAIL mediated apoptosis.43,44 Lexatumumab (HGS-ET2) is a human mAb that binds to and activates TRAIL-R2 and has been used in a phase I trial in adult solid tumors with minimal side effects.45 A phase I clinical trial in pediatric solid tumors including RMS, soft tissue sarcomas, osteosarcoma, Ewing’s sarcoma, hepatoblastoma and nephroblastoma was completed (NCT00428272).46 While some antitumor activity was evident, complete or partial responses were not realized. Considerations for combination and phase II studies are pending.

**Immunomodulatory mAbs**

**Cytotoxic T lymphocyte antigen 4 and programmed death receptor 1**

CTLA4 is a member of the immunoglobulin superfamily, expressed on the surface of T cells and transmits an inhibitory signal. T cell activation through the T cell receptor engagement and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules. CTLA4 is also found in regulatory T cells in which CTLA-4 blockade leads to their decreased immunosuppression. Programmed Death Receptor 1 (PD1) and its ligands (PD-L1 and PD-L2) are also part of the immune checkpoint pathway. In inflammatory environments PD 1 can decreases T cell activity, while in the tumor microenvironment PD1 ligand inhibits antitumor lymphocytes.47 PD1 is also highly expressed on T regulatory cells and when engaged by its ligand it is thought to enhance T regulatory proliferation.48 Iplilimumab, an anti-CTLA-4 antibody, in a phase III clinical trial of metastatic melanoma patients increased survival by 20%.49 The anti-PD1 antibody nivolumab has shown tumor responses in adult solid tumors including melanoma, lung cancer and renal cancers.50,51 The combination of CTLA-4 and PD1 antibodies are in phase I trials in multiple adult malignancies.52 The results of combination nivolumab and ipilimumab in stage III and IV melanoma was recently published.53 Patients randomized to nivolumab plus ipilimumab had an impressive increase in median progression-free survival to 11.5 mo compared to 2.9 mo with ipilimumab alone (p < 0.001).53 There are no clinical trials as of yet in pediatric solid tumors, however increased expression of PD1 has been found in patients with metastatic vs. localized osteosarcoma.54 In a mouse model of metastatic osteosarcoma combination of PD1 antibody and CTLA4 inhibitor lead to complete control of tumors.55 PD1
antibodies as well as CTL4A antibodies will likely be incorporated into phase I pediatric solid tumor clinical trials in the near future.

**CAR T cell adoptive cell transfer**

Use of CAR T cells for pediatric solid tumors has been challenging on several fronts. MHC molecules on tumors such as neuroblastoma are usually downregulated and epitopes for targeting are largely unknown for many pediatric tumors. Neuroblastoma was the first pediatric solid tumor in which CAR T cells have been tested in clinical trials. A phase I trial in patients with recurrent/refractory disease received Epstein Barr Virus (EBV) CTLs that were genetically modified to recognize GD2. Three of the 11 patients with active disease had a complete response and no significant toxicity was observed. A median follow-up of 96 weeks revealed low-level persistence of the GD2-CAR T cells and association with longer survival. Interestingly, patients who received the GD2-CAR T-cells did not experience significant pain as has been observed in anti-GD2 mAb therapy. There are ongoing GD2-CAR T cell clinical studies with a phase I trial in GD2+ solid tumors using escalating doses of anti-GD2-CAR infusions (NCT02107963). Third generation anti-GD2 CARs are undergoing phase I study for refractory neuroblastoma patients (NCT01822652). This GD-2-CAR integrates the CD28 and OX40 costimulatory endodomains with the hope of increasing persistence and anti-tumor effects. This CAR also contains an iCaspase suicide safety switch that can be activated leading to programmed cell death to prevent unanticipated toxicities such as cytokine storm. CAR T cell therapy directed against Her2/Neu has been used to target other pediatric solid tumors. The results of an ongoing phase I clinical trial in 19 patients with advanced pediatric sarcomas utilizing Her2/Neu-CAR T cells was recently published. Patients with HER2 positive refractory/recurrent sarcomas received escalating doses of Her2-CD28 T cells (NCT00902044). The cells persisted for six weeks without toxicities and responses were seen in some of the patients including four patients with stable disease. Further potential targets for CAR based therapy are currently under investigation for pediatric solid tumors.

**Anticancer vaccines**

While there is agreement that cancer vaccines are more effective in patients with minimal residual disease devoid of major immunosuppressive effects from T regulatory or myeloid suppressive cells, there is still no consensus for the “optimal” tumor vaccine. Vaccines derived from total tumor cells such as lysates, irradiated, genetically modified, apoptotic or necrotic tumor cells or tumor-derived chaperone proteins, allow for a wider array of antigens reducing the emergence of tumor escape variants, seen with single peptides, and do not require identification of specific antigens. In addition to the antigen component of the vaccine, an adjuvant is also essential. Adjuvants have ranged from attenuated bacterial products, emulsions such as Montanide or liposomal adjuvants, tensioactive agents such as saponins, alum and other minerals and cytokines such as GM-CSF.

**Peptide based vaccines**

An ongoing phase I/II trial of GD2 and GD3 antigens with OPT-821 adjuvant in combination with oral β-glucan recently enrolled 15 neuroblastoma patients in complete or very good partial remission. The vaccine contains GD2L and GD3L covalently linked to keyhole limpet hemocyanin (KLH). In the phase I component (NCT00911560) of 13 who completed the series of vaccinations, 12 remain relapse free at the time of publication (median of 32 mo) and 1 with a single node relapse. The Wilms tumor antigen (WT1) is expressed on many pediatric solid malignancies including nephroblastoma (Wilms tumor), neuroblastoma and RMS with. WT1 was ranked as the most promising tumor antigen by the NCI in 2009. It has been targeted in multiple trials in patients with leukemia. A Phase I/II trial was completed in patients with relapsed tumors with overexpression of WT1 protein and HLA-A*2402-positive. The HLA-A*2402 restricted, 9mer modified WT1 peptide was emulsified with Montanide ISA51 adjuvant and given intradermally to patients. The trial included four pediatric and young adult patients with sarcomas. The only adverse effect was injection site erythema. WT1 specific CTLs were found in three of the four patients with one having a complete response and one stable disease. WT1 may prove to be a useful target for pediatric solid tumors. As in adult cancers, the number of vaccine trials continues to grow with much anticipation of its role in solid tumor therapy.

**Dendritic cell based vaccines**

The largest dendritic cell vaccine trial in pediatric patients published to date included 30 patients with recurrent or metastatic Ewing’s sarcoma or alveolar RMS. Patients with confirmed t (11;22) or t(2;13) translocations had an initial cell harvest to collect autologous T cells and then received cytoreductive chemotherapy, radiation therapy and/or surgery. This was followed by infusion of autologous T cells and DCs pulsed with tumor specific peptides derived from tumor-specific breakpoints and E7 as a model antigen. Immune responses were seen in 39% of patients toward the tumor translocation breakpoint peptides, however responses were limited becoming undetectable six weeks later. The survival in the tumor vaccine group was 43% but it was difficult to ascertain the vaccine effect vs. historical control rates in this high risk population. There were no significant toxicities reported with the vaccine.

Multiple smaller phase I and pilot studies have been completed utilizing a variety of DC vaccines. A pilot study used a cancer vaccine in five patients with relapsed/refractory neuroblastoma or sarcoma post chemotherapy, radiation, and autologous peripheral blood stem cell transplant. Patients received autologous DCs pulsed with tumor specific synthetic peptides or tumor lysates. The tumor-specific peptides were synthesized after sequencing of the tumor-specific fusion transcripts. Delayed-type hypersensitivity (DTH) to the tumor was detected in all five patients. There was no significant toxicity and one of the neuroblastoma patients had stable disease for 27 mo and even more impressive a Ewing’s sarcoma patient had a complete response for 77 mo.
Active IL-12-secreting type 1 DCs vaccine was completed in pediatric and adolescent patients with a variety of refractory or metastatic solid tumors in a phase I study. Tumor tissue was obtained from patients a tumor cell lysate was prepared. Patients received a minimum of six treatments which included escalating doses of DCs and intact tumor cells irradiated with 120 Gy. Fourteen patients received subcutaneous and eight intranodal vaccine injections. Eleven of the fourteen patients also received human recombinant interferon gamma three times weekly with the cancer vaccine. The majority of patients did have a positive DTH test. No serious toxicities occurred and all patients given the vaccine intranodally were alive at the end of the trial as opposed to about half of the subcutaneously treated patients. However, the follow-up period was short (2–13 mo). The majority of patients did not have measurable tumor responses except for one patient with adrenocortical carcinoma who did achieve a partial response of lung metastases. The remaining patients demonstrated stabilization of disease. Another DC vaccine trial in refractory pediatric solid tumors utilized a similar vaccine. Immature DCs were combine with tumor cell lysates and KLH. Patients received DCs intradermally every two weeks for a total of three vaccinations. This trial again showed no serious adverse events and DTH response was found in seven of the ten patients that completed the immunization series. Regression of multiple metastatic sites was found in one patient and five patients had stable disease with a 16–30 mo follow up.

A phase I vaccine study in 11 neuroblastoma patients was conducted using DCs pulsed with tumor RNA after standard chemotherapy, surgery, radiation and high-dose chemotherapy with stem cell rescue. Of the three patients evaluated for tumor-specific response two demonstrated a response. One of these patients remained alive with stable disease 14 mo after diagnosis.

Cancer Testis Antigens (CTAs) are a group of antigens expressed on many tumor types including pediatric sarcomas and neuroblastoma. CTAs comprise 70 families with over 140 antigens. Their biologic function is not fully understood, but because of their immunogenicity they are being studied as T cell targets for vaccine and adoptive cellular therapy. However, not all antigens are immunogenic in all patients and expression of the antigens may vary between patients. There is an ongoing phase I study evaluating the use of decitabine with an autologous dendritic cell CTA specific (MAGE-A1, MAGE-A3, NY-ESO-1) vaccine for patients with refractory/recurrent sarcomas and neuroblastoma (NCT01241162). Decitabine is given initially as it can activate the use of decitabine with an autologous dendritic cell vaccine intranodally were alive at the end of the trial as opposed to about half of the subcutaneously treated patients. However, the follow-up period was short (2–13 mo). The majority of patients did not have measurable tumor responses except for one patient with adrenocortical carcinoma who did achieve a partial response of lung metastases. The remaining patients demonstrated stabilization of disease. Another DC vaccine trial in refractory pediatric solid tumors utilized a similar vaccine. Immature DCs were combine with tumor cell lysates and KLH. Patients received DCs intradermally every two weeks for a total of three vaccinations. This trial again showed no serious adverse events and DTH response was found in seven of the ten patients that completed the immunization series. Regression of multiple metastatic sites was found in one patient and five patients had stable disease with a 16–30 mo follow up.

Genetically modified tumor vaccines

Rousseau et al. initially conducted a phase I study in relapsed advanced neuroblastoma patients using an allogeneic neuroblastoma tumor cell vaccine combining lymphoactin with IL-2. Lymphoactin encourages lymphocyte chemotaxis and works synergistically with IL-2. The only adverse event was reversible panniculitis and bone pain. Measurable responses were detected in the majority of patients as well as complete remission in two and partial response in one. They followed this with another phase I trial in seven patients with recurrent neuroblastoma utilizing a tumor vaccine consisting of autologous instead of allogeneic neuroblastoma cells that were genetically modified to secrete IL-2 and lymphoactin with minimal toxicity. Tumor specific immune responses were measurable in five of six patients, and two of the seven patients had stable disease throughout the study. This group of investigators then went on to complete a phase I/II study in high-risk neuroblastoma utilizing autologous neuroblastoma cells genetically modified to secrete IL-2. Thirteen patients with limited tumor burden were enrolled consisting of those who had achieved a complete response, very good partial response or partial response to their initial therapy. There were no serious toxicities and median event-free survival was 22 mo for patients in first remission with four patients alive and three without disease recurrence. This study is being built upon with combining metronomic cyclophosphamide and adding additional tumor associated antigens (NCT01192555). A pilot study was recently published in 12 patients with advanced/metastatic Ewing’s sarcoma utilizing a tumor peptide vaccine, FANG. The vaccine has been utilized in multiple adult oncology studies and includes autologous tumor cells transfected with the rhGMCSF transgene and the RNAi biologics. There were no drug related toxicities in the 12 patients, 1 patient had a partial tumor response, and 8 patients remain alive to date of publication. The FANG phase I study for pediatric patients > 12 y of age is currently ongoing (NCT01061840) with plans for a phase II.

Viral based vaccines

Two phase I viral based vaccine reports in pediatric solid tumors were recently published. Pexa-Vec (JX-594), a viral vaccine derived from Wyeth vaccine strain with genetic GM-CSF gene insertion was tested in five patients with refractory/metastatic pediatric solid tumors. No patients had greater than grade 3 toxicity with the most common adverse event being fever. No patients had objective responses, however one patient with Ewing’s sarcoma had necrotic changes via MRI imaging of the injected tumor. Reovirus (Reolysin), a naturally occurring human virus that activates tumor lysis through the Ras pathway was evaluated in a phase I COG study in 29 patients with refractory/metastatic solid tumors. There were few toxicities notably hemato logic, fever, and mild transaminitis. Three patients had stable disease and received a second vaccination cycle, however there were no complete or partial responses noted. As these pilot trials have documented minimal toxicity
with some delay in tumor progression, additional studies are warranted to further evaluate efficacy in patients with minimal disease.

Conclusion

Pediatric cancer comprises only 1% of newly diagnosed cancers in the United States and 20% of those cancers are non-CNS solid tumors. Pediatric solid tumors that are metastatic or recurrent have a very poor survival which has not significantly changed over the past 50 y. Despite the small representation of pediatric solid tumors in oncology, the numerous pediatric basic and translation research reviewed herein is evidence of the optimism that immunotherapy may have a distinct role in the treatment of pediatric solid tumors. One of the primary reasons for the plethora of pediatric phase I and II trials for such a small population of patients is the acknowledgment of the value of these clinical trials by pediatric oncologists. The collaborative efforts of the COG as well as European Pediatric Groups have been the driving force behind many of these trials. The undeniable success of the anti-GD2 antibody dinutuximab in metastatic neuroblastoma is due to the ability of COG to organize meaningful large-scale immunotherapy clinical trials. This confirmed that immunotherapy is not only feasible but can improve the overall survival and quality of life of pediatric patients with solid tumors that carry a poor prognosis. Future clinical collaborative clinical trials in other solid tumors will continue to incorporate targeted immunotherapeutic strategies with more conventional regimens for a greater therapeutic impact in pediatric solid tumors.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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