Immunotherapeutic Challenges for Pediatric Cancers

Brian Hutzen,1 Mohammed Ghonime,1 Joel Lee,2 Elaine R. Mardis,1,2,3,4 Ruoning Wang,1,2,3 Dean A. Lee,1,2,3 Mitchell S. Cairo,5 Ryan D. Roberts,1,2,3 Timothy P. Cripe,1,2,3 and Kevin A. Cassady1,2,3,6

1The Research Institute at Nationwide Children’s Hospital, Center for Childhood Cancer and Blood Diseases, The Ohio State University College of Medicine, Columbus, OH, USA; 2The Ohio State University, Columbus, OH, USA; 3Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA; 4Institute for Genomic Medicine, The Ohio State University College of Medicine, Columbus, OH, USA; 5Department of Pediatrics, Cancer and Blood Diseases Center, New York Medical College, Valhalla, NY, USA; 6Division of Pediatric Infection Diseases, New York Medical College, Valhalla, NY, USA

Solid tumors contain a mixture of malignant cells and non-malignant infiltrating cells that often create a chronic inflammatory and immunosuppressive microenvironment that restricts immunotherapeutic approaches. Although childhood and adult cancers share some similarities related to microenvironmental changes, pediatric cancers are unique, and adult cancer practices may not be wholly applicable to our pediatric patients. This review highlights the differences in tumorigenesis, viral infection, and immunologic response between children and adults that need to be considered when trying to apply experiences from experimental therapies in adult cancer patients to pediatric cancers.

Tumors are an amalgamation of malignant cells, infiltrating stromal cells, and immune cells. Together, these malignant cells and non-malignant infiltrating cells can orchestrate a chronic inflammatory and immunosuppressive microenvironment that can restrict both oncolytic viral and immunotherapeutic approaches in some tumors.1–4 Although childhood and adult cancers share several commonalities, we are increasingly aware that pediatric cancers are unique and that the observations and lessons learned from the management of adult malignancies may not be wholly applicable to our pediatric patients. Pediatric cancers display lower mutation rates than their adult counterparts and are often driven by aberrant transcription or chromosomal rearrangement of genes involved in growth and development.5,6 Children, depending on their age and prior exposure, can also differ from adults in terms of their immune response, and this may influence their response to cancer immunotherapy.

While children outside of the neonatal period are not immunocompromised, they undergo immune development in the initial years of life. Given similar antigenic stimuli, children generate lower type I and II interferon responses compared to adults and indeed may be unable to respond to certain antigenic stimuli (e.g., polysaccharide antigens) altogether prior to reaching a certain age. This is due in part to ontologic differences in their response and to reduced memory cell populations related to less antigenic exposures. This review highlights the differences in tumorigenesis, viral infection, and immunologic response between children and adults that need to be considered when trying to apply experiences from experimental therapies in adult cancer patients to pediatric cancers.

Pediatrics: Differences in Tumor, Immune, and Anti-viral Mechanisms Compared to Adults

Adult Solid Tumors and Immunosuppressive Mechanisms

Immunosuppressive Microenvironment. At the superficial level, pediatric solid tumors are similar to their adult counterparts: they exhibit significant myeloid and stromal cell infiltration, display chronic nuclear factor κB (NF-κB) and signal transducer and activator of transcription (STAT)-mediated chemokine, cytokine, and growth factor production, and generally maintain an immunosuppressive microenvironment.1,8–10 Although the composition of cellular infiltrates can vary across different tumor types, some of the most prominent infiltrating cells are tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). TAMs derive from myeloid precursors in the bone marrow and play profound and diverse roles in tumors both through direct contact and paracrine effects that impact/ regulate tumorigenesis, vasculogenesis, tumor cell growth, extracellular matrix deposition/remodeling, and response to therapy (see reviews11,12). Macrophages are recruited to the tumor though the process of chemotaxis.

Whereas macrophages activated by interferon gamma (IFNγ) are generally tumoricidal, most TAMs are polarized by interleukin (IL)-4 or IL-13 in combination with IL-10 and colony-stimulating factor (CSF) 1. These cytokines and other tumor-associated factors polarize macrophages toward tissue repair and remodeling functions, wherein they secrete angiogenic, proliferative, and immunosuppressive factors (e.g., vascular endothelial growth factor A [VEGF-A], IL-8, tumor necrosis factor alpha beta [TNF-αβ], matrix metalloproteinase 9 [MMP-9], and transforming growth factor β [TGF-β]), which promote tumor progression.13 Several studies suggest TAMs are...
abundant in pediatric solid tumors. In a histologic study of a panel of human samples, Maris’ group found that many types of pediatric cancers stained positively for macrophages, notably Burkitt lymphoma (10/10, 100%), osteosarcoma (14/20, 70%), neuroblastoma (38/118, 32%), Ewing sarcoma (11/25, 44%), and rhabdomyosarcoma (15/53, 28%). Gorlick and colleagues reported similar findings in osteosarcoma, where TAM presence conferred a poor prognosis.

Like TAMs, MDSCs originate from myeloid precursors in the bone. Typically, infections stimulate the production of granulocytic MDSCs (G-MDSCs, also known as polymorphonuclear MDSCs), whereas tumors will generally stimulate the production and recruitment of myeloid MDSCs (M-MDSCs) through the secretion of a number of soluble factors. Both G-MDSCs and M-MDSCs produce immunosuppressive factors like IL-10, prostaglandin E2 (PGE2), and TGF-β. These myeloid infiltrates, together with CD4+FOXP3+ regulatory T cells (Tregs), can markedly inhibit cytotoxic T lymphocyte (CTL) proliferation and activation. The chronic cytokine and chemokine production of tumor and stromal cells creates a microenvironment that can paradoxically be considered both inflamed and immunosuppressed. T cells in this microenvironment, which are also subject to chronic tumor antigen stimulation, can rapidly become exhausted and rendered ineffective. These features are particularly prevalent in therapeutically resistant pediatric solid tumors, such as gliomas, sarcomas, and poor prognosis neuroblastoma, but also in resistant adult cancers, such as pancreatic cancer, carcinomas, and ovarian cancers.

**Metabolic Derangements.** The pediatric solid tumor microenvironment, like adult tumors, also exhibits extreme metabolic changes that contribute to tumor growth and immunosuppression. A hallmark of malignant cells from adults and children is heightened nutrient consumption, which results in the intratumoral depletion of carbon and nitrogen source (glucose and glutamine), and the release of immunosuppressive metabolites, such as lactate acid, kynurenine, adenosine in response to hypoxia, acidity, and other stressors. Tumor-associated CD39 (also called NTPDase1) and CD73 (ecto-5’-nucleotidase) collaborate to degrade ATP and increase extracellular adenosine where it aids proliferation, angiogenesis, and antitumor immunosuppression. As such, solid tumors develop a microenvironment that is hostile to infiltrating immune cells, where the metabolically demanding cancer cells restrict the function of CTLs by competing for nutrients and by producing immunosuppressive metabolites: a phenomenon known as metabolic antagonism.

**Pediatric Tumors and Transcriptionally Driven Responses**

While pediatric cancers share some characteristics with adult solid tumors (as described above), they also differ fundamentally from adult tumors in others. First, pediatric cancers more often arise from distinct oncogenic mechanisms than do most adult cancers. Children, by virtue of their age, have experienced fewer cell division cycles and have less background mutations than adults. Furthermore, they have had less exposure to the environmental factors and genotoxic stressors that contribute to many adult malignancies. Many pediatric cancers instead either appear to be rooted in germline alterations that predispose the child to cancer or the consequence of chromosomal rearrangements that can activate proto-oncogenes, silence tumor suppressors, or create chimeric oncoproteins that act as aberrant transcription factors.

Several fusion proteins have been identified in pediatric malignancies and are commonly used as disease/prognostic markers, and in some cases they may also provide therapeutic targets. One of the best known examples in pediatric solid cancers is the reciprocal translocation of chromosomes t(11;22)(q24;q12) found in 85% of Ewing sarcoma tumors. This translocation joins the amino terminus of the EWS gene and the carboxy terminus of the FLI1 gene, producing an aberrant transcription factor that drives tumorigenesis. Selective blockade of the EWS-FLI1 transcription factor was shown to sensitize Ewing sarcoma tumors to chemotherapy, and novel therapies designed to target EWS-FLI1 protein-binding interactions or to reverse its transcriptional signature are the focus of several ongoing clinical trials.

Other notable examples of chromosomal abnormalities in pediatric solid tumors are the closely related translocations of t(2;13)(q35;q14) and t(13;12)(p36;q14) found in 55% and 22% of alveolar rhabdomyosarcomas, respectively. These translocations juxtapose the DNA-binding domain of PAX3 or PAX7 with the activation domain of FKHR, and they are thought to induce tumorigenesis by abrogating the differentiation of normal skeletal muscle cells. Although the pursuit of developing therapies centered on pediatric fusion drivers has merit, their impact on the transcriptional program is limited by several factors, including the difficulty of therapeutically inhibiting transcription factors.

**Tumor Antigens and Immune Evasion.** Tumor-associated antigens (TAs) are short polypeptides that originate from proteasome-mediated degradation of proteins from cancer cells during autophagy or following their engulfment by phagocytic cells. These newly produced peptides are transported from the cytosol into the lumen of the endoplasmic reticulum by the transporter associated with antigen processing (or TAP) complex, where they are loaded onto nascent major histocompatibility complex class I (MHC class I) molecules. TAA-bearing MHC class I molecules are then transferred to the cell surface, where they can subsequently activate CTLs and provoke an antitumor immune response.

Impaired antigen presentation, either through the downregulation of MHC class I or other components of the antigen presentation machinery, is a key mechanism through which cancer cells can circumvent immune surveillance, and it poses a particular challenge for treating childhood cancers. Despite their similarities, the etiology of pediatric solid tumors is considerably different than that of adults. In particular, the majority of pediatric cancers arise from embryonal cells rather than epithelial cells, and they are thought to result from transcriptional abnormalities rather than the gradual accumulation of genetic mutations over time. As such, pediatric tumors harbor
significantly lower mutational burden and express fewer potential neoantigens, limiting their susceptibility to immune targeting. Although the relative paucity of neoantigens is a defining trait of pediatric tumors, it is not uncommon for them to express membrane-associated embryonic antigens (e.g., EphA2) or novel transcription factors created by chromosome rearrangements. While these perturbations often have a hand in driving tumor proliferation, they may also provide potential immunotherapeutic targets.56–58

VirotHERAPy: ChilDren versus AduLts

Oncolytic virotherapy is an emerging class of cancer therapeutics that integrates virus-induced tumor cell cytotoxicity, immune cell recruitment, and the induction of an antitumor immune response against these antigens. Both viral replication and the immune-mediated response contribute to antitumor efficacy.59,60 Viral infections are well described at breaking immune tolerance and inducing auto-immunity to self-antigen.61–64 By recruiting and stimulating immune activity and tumor cell damage, oncolytic viruses provide an opportunity to prime immune recognition of neoantigens or to break immune tolerance to fetal proteins expressed by the tumor. To date, the vast majority of pre-clinical and clinical studies conducted with oncolytic viruses have focused on adult malignancies. While the information gleaned from these studies has been invaluable for the progression of the field, children are not simply small adults, and the assumptions based on adult patients may not necessarily apply to pediatric patients.

Age is a major determinant for susceptibility to viral infection and manifestation of disease in children. Children exhibit differing disease susceptibility and pathogenesis from viral infections than adults. In a series of classical studies, Diane Griffin and colleagues57,65 exposed neonatal and pediatric mice to Sindbis virus to compare viral replication rates and examine immune-mediated effects associated with infection. Neonatal mice invariably died of encephalomyelitis within a few days of Sindbis infection, whereas older mice were able to clear the virus without signs of paralysis or neurological damage.66,67 Susceptibility in this case was not associated with maturation of the immune response, but rather with the differing susceptibilities of mature and immature neurons to Sindbis infection. Mature neurons were shown to be intrinsically more resistant to Sindbis virus replication than their immature counterparts, which were subject to virus-induced apoptosis. It should be noted, however, that even though pediatric mice were resistant to initial infection, their complete recovery was ultimately dependent on immune-mediated clearance of the remaining virus.

Outside of the neonatal period, children are not considered immunocompromised; however, there are subtle differences in their developing immune response. Some viral infections are better tolerated and lead to less immune-mediated disease when they occur at a younger age. For example, Epstein-Barr virus and hepatitis A infection in children are often asymptomatic in younger children (<6 years of age) but produce immune-related symptoms in adolescents and adults.70–71 Much of this difference in susceptibility to infection and disease relates to developmental differences in the immune response between children and young adults. In contrast, other infections (e.g., enteroviral and adenovirus infections) in the initial weeks of life or in premature infants produce high mortality rates.72–75 Other infections like the mosquito-borne La Crosse virus produces encephalitis more frequently in children <12 years of age, whereas older children develop non-CNS manifestations of the disease.76

Children are born in a relatively immunocompromised state and are highly susceptible to infection in the initial months of life.77–79 They have reduced intrinsic (e.g., immunoglobulin [Ig] and complement), innate (e.g., polymorphonuclear neutrophil [PMN] and macrophage), and adaptive immune responses.80 Outside of the neonatal period, children are no longer considered immunocompromised; however, their T cell and antigen presentation activity develop over the initial years of life, and this can influence their vaccine response and susceptibility to infection and disease.81,82 Depending upon their age, infants, toddlers, school-age children, and young adults can differ in their lymphoproliferative and antibody responses.81,83,84 Furthermore, children less than 4 years of age (and even toddler mice) generate reduced pro-inflammatory cytokines in response to antigenic stimuli than adults, such as type I IFNs, IFNγ, and IL-12, and they can exhibit a weaker T cell response to some infections.78,85

These differences in the immune response of children coupled in many cases with a lack of prior immunity can alter replication and viral clearance kinetics in the pediatric setting.85–88 Even so, early studies by our co-author (T.P.C.) have shown that these therapies are safe in pediatric patients. While the prospect of more robust oncolytic virus replication may appear beneficial for treating pediatric tumors, differences in the intrinsic and adaptive immune responses in pediatric patients to this type of therapy are less well characterized due to the limited number of clinical trials. The perceived benefits of increased oncolytic virus replication in this setting thus may be offset by a decreased antitumor immune response, but further testing is certainly needed.

Viro-Immunotherapy and Cellular Therapies

Viro-immunotherapy

Oncolytic virotherapy is an immunotherapeutic cancer treatment approach that involves a multistep process of direct tumor cell lysis followed by the induction of cytotoxic or apoptosis-sensitizing cytokines and the promotion of immune-mediated antitumor responses.89 Both investigators and pharmaceutical companies are actively pursuing attenuated oncolytic viruses as novel therapeutic agents.90–102 These viruses are species specific or engineered with attenuating gene modifications that restrict infection in non-malignant human cells but that are dispensable in cancer cells. While several oncolytic virus platforms exist and each arguably has its own merits, few direct comparisons have been performed between different viral platforms and their relative efficacy in different tumor types.

We and others have shown that tumor models can differ greatly in their therapeutic responsiveness.103–111 Previous studies have shown that early generation viruses that were conservatively designed for
safety were often unable to replicate effectively in these tumor cells, and this likely hampered their ability to elicit a sustained antitumor immune response.\textsuperscript{104,114} To overcome this shortcoming, we and others have engineered next-generation viruses with improved replication in tumor cells or viruses that activate immunostimulatory pathways or carry transgenes that stimulate different arms of the antitumor immune response.\textsuperscript{106,115–117} Based on early clinical successes, current strategies to target more resistant tumor types involve combining virotherapy with immunomodulatory agents, such as immune checkpoint inhibitors or agents that alleviate tumor-associated immunosuppression to extend viro-immunotherapeutic activity.\textsuperscript{60,90,118–120} Preliminary results suggest that this is highly effective in some tumor types. This strategy is alluring as it can utilize well-characterized oncolytic viruses that have already undergone extensive safety and efficacy analysis as single-agent therapies. Based on initial tumor biomarker analysis and immune recruitment, adjuvant immunomodulatory therapies (e.g., Ig anti-PDL1, Ig anti-CTLA2, Ruxolitinib, and STING agonists) can then be rationally selected and incorporated into follow-up dosing regimens to improve patient responses.

**Cellular Therapies**

The field of cancer immunotherapy has witnessed a renaissance, as we have expanded our knowledge of the immune system and our ability to manipulate it with therapeutic intent. This has largely been advanced through approaches involving the adaptive arm of the immune system with such modalities as monoclonal antibodies, vaccines, antigen-specific T cells, and immune checkpoint blockade. Cellular therapies are advancing: antibody-targeted therapy is the standard of care for some malignancies. Immune checkpoint blockade has become frontline therapy for carcinomas, and a single dose of genetically modified T cells can be curative for CD19+ B cell malignancies.\textsuperscript{121–124} Solid tumors, however, have been less responsive to immunotherapeutic approaches.\textsuperscript{125,126} Furthermore, single-antigen targeting (using monoclonal antibodies or genetically modified chimeric antigen receptor [CAR] T cells) has been limited to a few diseases bearing special-case antigens, and their restricted antigenic repertoire is prone to failure by immune escape.\textsuperscript{127,128} Lastly, pediatric cancers have been less responsive to approaches that depend on endogenous immunity to broader antigenic repertoires because of their inherently low mutational burden.

**CAR Therapy**

The recent development and success of CAR T cell therapy in childhood acute lymphoblastic leukemia has renewed interest in this immunotherapy approach for other poor risk malignancies.\textsuperscript{129–131} CAR T cells usually contain a single-chain variable fragment from a monoclonal antibody; a transmembrane hinge region; and a signaling domain such as CD28, CD3ζ, or 4-1BB.\textsuperscript{128,132,133} The advantage of the CAR strategy is that no HLA expression on the target cell is required for the epitope to be accessible to CAR+ immune cells. However, use of CAR T cells can produce a life-threatening cytokine release syndrome (CRS), CAR T cell-related encephalopathy syndrome (CRES), and sustained on-target off-tissue effects.\textsuperscript{124,125}

**NK-Based Cellular Therapies**

Natural killer (NK) cells represent the human body’s first line of defense against tumor cells and infectious pathogens, and they play a key role in tumor immunosurveillance. NK cells are a key component of antitumor immunity that are characterized by their ability to recognize and kill tumor targets in an antigen- and MHC-unrestricted manner, and they elicit a pro-inflammatory environment that primes adaptive immunity. Phenotypically, NK cells are lymphocytes that lack B and T cell markers CD19/TCR/CD3 on their cell surface, but they express CD16 and/or CD56 surface antigens. NK cells are further characterized by the degree of CD56 expression into dim and bright subsets, which have significant differences in terms of cytokine production, response to cytokines, and their killing potential.\textsuperscript{136}

NK cells do not require prior sensitization to target transformed malignant cells.\textsuperscript{137} NK cells recognize autologous cells that express human leukocyte antigen (HLA) class I molecules that prevent them from attacking the host tissue, known as tolerance to self. NK cells also have the unique ability to exert antibody-dependent cell-mediated cytotoxicity (ADCC) due to the presence of Fc receptor FcγRIIIa that recognizes the Fc portion of the antibodies. There are several mechanisms by which NK cells can kill the target cells.\textsuperscript{138} They can exert direct cytoxicity through the release of granules containing perforin and granzyme.\textsuperscript{139} In addition, they can mediate cytoxicity via apoptotic pathways involving Fas ligand or TNF-related apoptosis-inducing ligand (TRAIL).\textsuperscript{139–141}

In contrast to T cells, NK cells have not been associated with significant off-target effects, graft-versus-host disease (GVHD), or CRS. NK cell number and function are low in patients with tumors; traffic poorly to tumor sites or across the blood-brain barrier; and are further depleted by chemotherapy, radiation, and surgical anesthesia. This provides a strong rationale for restoring NK cell function through adoptive transfer. Adoptive immunotherapy overcomes the problem of low NK number and function, and it overcomes the shortcomings of antigen-specific approaches by broadly recognizing danger- and stress-related proteins while simultaneously activating adaptive immunity through multiple mechanisms that increase antigen recognition. NK cells have shown significant alloreactive anti-leukemic effects against liquid tumor cells, especially following haploidentical stem cell transplantation (SCT).\textsuperscript{142} and higher NK cell immune reconstitution in the early post-allogeneic SCT period has been associated with significantly improved survival and lower leukemia relapse rates.\textsuperscript{143,144}

The addition of NK cells to haploidentical transplant for high-risk myeloid malignancies is showing unprecedented results in ongoing phase II studies, with 1-year relapse rates of less than 10%. However, NK cell resistance to solid tumors is in large part due to the small numbers of active NK cells and lack of specific tumor targeting of NK cells. Until recently, NK cell therapy has lagged behind other approaches due to insufficient methods to generate large numbers of active NK cells for adoptive transfer. Now there is a robust approach for generating large numbers of clinical-grade NK cells, which have...
since been delivered to adults and children with cancer.\textsuperscript{145-147} Thus far, over 80 subjects (including pediatric patients with leukemia, brain tumors, and neuroblastoma) in several ongoing trials have received over 200 infusions of these cells.

**Dendritic Cell Vaccines**

Dendritic cells (DCs) are the most powerful antigen-presenting cells (APCs), capable of stimulating naive and memory CD8\(^+\) T cells as well as CD4\(^+\) helper T cells, and they have a pivotal role in the regulation of immune responses. Preliminary findings in murine studies showed that DCs pulsed with tumor extracts can significantly delay cancer growth,\textsuperscript{148} result in humoral and cellular immune responses,\textsuperscript{149} and confer \textit{in vivo} resistance to tumor challenge.\textsuperscript{150} DC vaccines have generally been made of autologous monocytes that are differentiated and matured \textit{in vitro} by treatment with granulocyte-monocyte colony-stimulating factor and IL-4 and pulsed with antigen before injection. Sipuleucel-T is the only DC vaccine that has shown sufficient efficacy in a phase III clinical trial to gain FDA approval. It is an autologous DC vaccine primed with a recombinant antigen composed of prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant.\textsuperscript{146} There have been a number of early clinical trials evaluating DC-based vaccines in the therapy of cancer in adult patients with melanoma, renal cell cancer, and prostate cancer.\textsuperscript{151-153} Geiger et al.\textsuperscript{154} showed that, in pediatric malignancies, it is not only feasible to generate DC in children, including those as small as 13 kg, but also DC-based vaccines can be administered in an outpatient setting without significant toxicity. More than 30 clinical trials are currently open that include DC-based immunotherapy in pediatric and adult cancers.\textsuperscript{155}

**Clinical Trial Experience in Children**

**Virotherapy**

At the time of this writing, only a few clinical trials have investigated the use of oncolytic virotherapy in a pediatric setting. The Children’s Oncology Group conducted a phase I trial wherein 24 children with relapsed or refractory extracranial solid tumors were treated with intravenous administration of the oncolytic reovirus Reolysin (ClinicalTrials.gov: NCT01240538).\textsuperscript{156} While the virus was well tolerated and no dose-limiting toxicities were reported, no objective responses were seen, and the authors concluded that efficacy would be dependent upon combining reovirus with other therapeutics.\textsuperscript{157} The Children’s Oncology Group also recently conducted a study of intravenously administered Seneca Valley virus in 22 children with rhabdomyosarcoma, relapsed neuroblastoma, or other tumors with neuroendocrine features (ClinicalTrials.gov: NCT01048892), and, while the dosing levels they employed were tolerable, the virus was quickly neutralized and there were no objective responses.\textsuperscript{158} Similarly, intratumoral administration of a vaccinia virus, JX-594, or of an oncolytic herpes simplex virus (oHSV), Seprehvir, was found to be safe in children, adolescents, and young adults, but there were no objective responses at the doses tested.\textsuperscript{150,159}

Another clinical trial being conducted at the University of Alabama at Birmingham seeks to determine the safety of an oHSV known as G207 to treat children with recurrent or progressive supratentorial brain tumors (ClinicalTrials.gov: NCT02457845).\textsuperscript{160} Other viruses with less conservative designs are also entering clinical trials for brain tumors in children. Having completed safety studies in adults, a recombinant polio/rhinovirus (PVSRIPO) has entered early stage clinical trial in pediatric patients (ClinicalTrials.gov: NCT03043391). In addition, the Pacific Neuro-oncology Consortium (PNOC) has an ongoing phase I study using a modified measles virus for the treatment of children and young adults with recurrent medulloblastoma or recurrent atypical teratoid rhabdoid tumors (ATRTs).

Amgen is also testing intratumoral injection of Talimogene laherparevvec (also known as T-VEC or Imlygic) in pediatric patients with solid tumors (ClinicalTrials.gov: NCT02756845). T-VEC is an oHSV that was recently approved by the FDA for intralesional injection of patients with melanoma.\textsuperscript{161} This virus is deleted for the aforementioned γ134.5 and a virus protein known as ICP47 or Us12, which normally suppresses peptide loading onto HLA class I and thus helps the virus evade an adaptive immune response.\textsuperscript{162} Spontaneous and engineered deletion of the Us12 gene also mutates the native Us11 late promoter.\textsuperscript{163,164} This juxtaposes the Us12 alpha promoter with the Us11 gene, creating an alpha Us11 and enabling the virus to evade protein kinase R (PKR) translational arrest and other double-stranded RNA (dsRNA)-activated pathways.\textsuperscript{164,165} T-VEC also encodes a transgene for GM-CSF to help potentiate immune reactions. This study is now open and enrolling (ClinicalTrials.gov: NCT02756845). One of our co-authors (T.P.C.) is also involved in a phase I trial to evaluate the safety of image-guided injection of HSV1716 in young patients with relapsed or refractory extracranial cancers (NCT00931931).

Thus far, 18 patients have been treated with different dose levels in intratumoral\textsuperscript{166} and intravenous studies. There is evidence of virus replication and inflammatory reactions in tumors from both sets of patients, but no indications of virus-related serious adverse events. While we observed several cases of stable disease, we did not detect any tumor regressions. Due to feasibility issues, the highest virus dose tested was 10-fold less than what was used in the trials that led to the approval of T-VEC. Nevertheless, with these safety data in hand, we are emboldened to test higher doses of oncolytic viruses in future studies and include rational combinations based on pre-clinical data.\textsuperscript{157}

**Cellular Therapy Experience**

Research activities investigating different cellular therapies for pediatric malignancies have been bolstered by recent success using CAR T cell therapy in childhood acute lymphoblastic leukemia. This has renewed interest in a cellular immunotherapy approach for other poor risk malignancies.\textsuperscript{36-38} Immunotherapeutic approaches are now focusing on solid tumors and other therapeutically resistant cancers. At the time of this writing, there are a growing number of CAR-, autologous NK cell-, and DC vaccine-based pediatric trials ongoing. These are summarized in Table 1. Past NK cell translational...
approaches were limited by the paucity of NK cell numbers available to infuse. This issue has been remedied by the discovery of methods to successfully expand NK cells \textit{ex vivo} (e.g., using IL-21-expressing cell lines), leading to an increase in NK-related studies.\textsuperscript{145} Now efforts are focused on enhancing their function through adjuvant therapies or epigenetic modification using cytokines. NK cells and DC vaccination approaches are also being incorporated into stem cell transplant protocols to target residual disease and in an attempt to reduce GVHD. Other investigators are using vaccination approaches based to prime an antitumor immune response against cancer antigens, using total tumor-associated RNA (e.g., ClinicalTrials.gov: NCT01326104) or autologous DC peptide vaccines.

We searched \url{https://clinicaltrials.gov/} using the terms pediatric cancer + natural killer chimeric antigen receptor or dendritic cell; apologies if we overlooked your study.

### Table 1. Clinical Trials Involving Chimeric Antigen Receptor or NK Cellular Therapy Actively Recruiting at the Time of this Writing

| ClinicalTrials.gov ID | Description                                                                 | Intervention                                      | Tumor                                      |
|-----------------------|------------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------|
| NCT02932956           | CAR T cells against Glypican-3-expressing pediatric solid tumors             | CAR T cell                                       | Glypican-3-expressing solid tumors (liver)  |
| NCT03500991           | CAR T cells against HER2[+] pediatric CNS tumors                             | CAR T cell                                       | HER2[+] pediatric CNS tumors               |
| NCT03638167           | EGFR-specific CAR T cell for CNS tumors                                      | CAR T cell                                       | EGFRvIII gliomas and CNS tumors            |
| NCT02315612           | CART T cells against CD22-expressing B cell malignancies                    | CAR T cell                                       | CD22-expressing B cell malignancies        |
| NCT02772198           | CAR T cells against CD19-expressing B cell malignancies (ALL and NHL)       | CAR T cell                                       | CD19-expressing malignancy                |
| NCT01555892           | CAR T cells against EBV-LMPI, BRAF, and EBNA(+) tumors                      | CAR T cell                                       | lymphomas and carcinomas                  |
| NCT02892695           | bridge immunotherapy in unresponsive CD19(+) leukemia lymphoma               | CAR-NK+ HSCT                                      | leukemia or lymphoma                       |
| NCT02573896           | expanded autologous NKS + chimeric IgG GD2 (CH14.18) + lenalidomide         | autologous NK                                    | refractory and relapsed neuroblastoma      |
| NCT02650648           | expanded autologous NK + humanized IgG GD2 (Hu3F8)                          | autologous NK                                    | refractory and relapsed neuroblastoma      |
| NCT03209869           | expanded autologous NKS + chimeric IgG GD2 (CH14.18) + rIL2                | autologous NK                                    | refractory and relapsed neuroblastoma      |
| NCT03420963           | chemotherapy (cyclophosphamide, etoposide, mesna) + NK cells                | autologous NK                                    | pediatric solid tumors                     |
| NCT02100891           | allogeneic HCT + NK cells in pediatric solid tumors: phase II               | autologous NK                                    | sarcomas, neuroblastoma, and CNS tumors    |
| NCT01823198           | NK cells with HLA-compatible HCT for high-risk myeloid malignancies          | autologous NK                                    | AML/myeloproliferative disease             |
| NCT01904136           | phase I/II trial of NK cell administration to prevent relapse for high-risk myeloid malignancies undergoing allogeneic stem cell transplantation | autologous NK                                    | AML/myeloproliferative disease             |
| NCT02809092           | phase I/II trial of IL-21-expanded NK cells for relapsed/refractory acute myeloid leukemia induction therapy | autologous NK                                    | AML/myeloproliferative disease             |
| NCT01898793           | phase 1/2 study of cytokine-induced memory-like NK cells in patients with AML or MDS | autologous NK                                    | AML/myeloproliferative disease             |
| NCT01619761           | natural killer cells in allogeneic cord blood transplantation                | autologous NK + CB                               | poor risk hematologic malignancies         |
| NCT01326104           | vaccine immunotherapy for recurrent medulloblastoma (phase II DCs) and PNETs (phase I lymphocytes) | tumor-RNA primed DCs or autologous lymphocytes  | medulloblastoma and peripheral neuroectodermal tumors (PNETs) |

We searched \url{https://clinicaltrials.gov/} using the terms pediatric cancer + natural killer chimeric antigen receptor or dendritic cell; apologies if we overlooked your study.

**Conclusions**

As cancer immunotherapy has gained traction, innovative approaches are being devised to address the immune system-related challenges in the pediatric setting and build upon the success of CAR T in B cell acute lymphoblastic leukemia (ALL). These approaches are being developed in recognition of the differences and similarities between pediatric and adult cancers and the corresponding immune systems. They are furthermore being buttressed by basic science efforts to better evaluate the transcriptional drivers in pediatric cancers, as well as the corresponding changes to transcriptional control elicited by novel, fusion-created transcription factors; by chromatic rearrangement in response to histone mutations; or by...
other epigenetic phenomena that set the stage for cancer development. By examining these downstream, pathway-based influences, new information may be gained regarding therapeutic interventions or novel immune targets that, for example, utilize the innovations outlined in this review.

In broad strokes, immunity comprises two interconnected branches—innate and adaptive. Both branches extensively utilize cellular and humoral mechanisms to recognize infection and tumor cells. Currently approved cancer immunotherapies rely on tumor-infiltrating lymphocytes, vaccines, viral therapeutics, checkpoint inhibitors, and CARs that target cell surface and MHC-restricted antigens. However, these approaches rely on high tissue specificity, low off-tissue toxicity, or high mutational burden, which have been uncommon in pediatric cancers other than B cell malignancies. The biggest success of immunotherapy for childhood cancers thus far (CAR T) involved targeting a normal cell surface protein. Pediatric cancers have lower mutational burdens and fewer neoantigens, and, therefore, they may be less responsive than adult tumors to pure adaptive immunotherapeutic approaches. Instead, pediatric cancers may require complementary approaches using less specific immunotherapeutic agents that both create a pro-inflammatory environment and/or break immune tolerance to leverage and prime innate and adaptive immune cells.

CONFLICTS OF INTEREST
The authors in full transparency disclose the following commercial interactions; however, we do not anticipate that they pose a significant conflict for this invited review on pediatric immunotherapy. (1) E.R.M.: PACT Pharma LLC, scientific advisory board, honorarium, and stock options; Moderna, Inc., scientific advisory board, honorarium; Regeneron Genomics Center, scientific advisory board, honorarium; (2) D.A.L.: CytoSen Therapeutics, cofounder, scientific advisory board, stock; Courier Therapeutics, advisory board; Merck Sharp and Dohme, consultant; Caribou Biosciences, advisory board; (3) K.A.C.: stock, Mustang Bio licensure; and (4) M.S.C.: Jazz Pharmaceuticals, advisory board, speaker bureau; Nektar, advisory board; Celgene, advisory board.

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