Molecular Therapy
Oncolytics
Review

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. Cassady[1,2,3,6]

[1]The Research Institute at Nationwide Children's Hospital, Center for Childhood Cancer and Blood Diseases, The Ohio State University College of Medicine, Columbus, OH, USA; [2]The Ohio State University, Columbus, OH, USA; [3]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA; [4]Institute for Genomic Medicine, The Ohio State University College of Medicine, Columbus, OH, USA; [5]Department of Pediatrics, Cancer and Blood Diseases Center, New York Medical College, Valhalla, NY, USA; [6]Division 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.[5–7] 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., polysacharride 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 reviews[11,12]). 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 metallopeptidase 9 [MMP-9], and transforming growth factor β [TGF-β]), which promote tumor progression.[13] Several studies suggest TAMs are

https://doi.org/10.1016/j.omto.2019.08.005.

Correspondence: Kevin A. Cassady, MD, The Research Institute at Nationwide Children's Hospital, Center for Childhood Cancer and Blood Diseases, The Ohio State University College of Medicine, 700 Children's Drive, Columbus, OH 43205, USA.
E-mail: kevin.cassady@nationwidechildrens.org

abundant in pediatric solid tumors. In a histologic study of a panel of human samples, Maris' group[14] 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[15] 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.[16] Both G-MDSCs and M-MDSCs produce immunosuppressive factors like IL-10, prostaglandin E2 (PgE2), and TGF-β.[16,17] These myeloid infiltrates, together with CD4+FOXP3+ regulatory T cells (Tregs), can markedly inhibit cytotoxic T lymphocyte (CTL) proliferation and activation.[18] 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.[8,19] 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.[20–25]

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 lactic acid, kynurenine, adenosine in response to hypoxia, acidity, and other stressors.[26–37] 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.[35,38,39] 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.[28]

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.[40]

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.[41,42] 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.[43,44] 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.[45–48]

Other notable examples of chromosomal abnormalities in pediatric solid tumors are the closely related translocations of t(2;13)(q35;q14) and t(1;13)(p36;q14) found in 55% and 22% of alveolar rhabdomyosarcomas, respectively.[49,50] 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.[51–53] 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 (TAAs) 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 1 molecules are then transferred to the cell surface, where they can subsequently activate CTLs and provoke an antitumor immune response.[54]

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.[55] As such, pediatric tumors harbor

significantly lower mutational burden and express fewer potential neoantigens, limiting their susceptibility to immune targeting.[5] 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.[61–66] 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 colleagues[67,68] 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.[69,70] 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.[59,71] 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.)[89] 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.[90] Both investigators and pharmaceutical companies are actively pursuing attenuated oncolytic viruses as novel therapeutic agents.[91–102] 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.[103–113] 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.[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.[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.[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.[121–124] Solid tumors, however, have been less responsive to immunotherapeutic approaches.[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.[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.[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.[129,132,133] 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.[134,135]

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.[136]

NK cells do not require prior sensitization to target transformed malignant cells.[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.[138] They can exert direct cytotoxicity through the release of granules containing perforin and granzyme.[139] In addition, they can mediate cytotoxicity via apoptotic pathways involving Fas ligand or TNF-related apoptosis-inducing ligand (TRAIL).[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),[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.[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.[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,[148] result in humoral and cellular immune responses,[149] and confer *in vivo* resistance to tumor challenge.[150] DC vaccines have generally been made of autologous monocytes that are differentiated and matured *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.[148] 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.[151–153] Geiger et al.[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.[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).[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 therapies.[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.[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.[89,159]

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).[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 1 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 laherparepvec (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.[161] This virus is deleted for the aforementioned $\gamma_1$34.5 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.[162] Spontaneous and engineered deletion of the Us12 gene also mutates the native Us11 late promoter.[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.[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[89] 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.[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.[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

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-LMP1, 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 IgαGD2 (CH14.18) + lenalidomide	autologous NK	refractory and relapsed neuroblastoma
NCT02650648	expanded autologous NK + humanized IgαGD2 (Hu3F8)	autologous NK	refractory and relapsed neuroblastoma
NCT03209869	expanded autologous NKs + chimeric IgaGD2 (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 https://clinicaltrials.gov/ using the terms pediatric cancer + natural killer chimeric antigen receptor or dendritic cell; apologies if we overlooked your study.

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 *ex vivo* (e.g., using IL-21-expressing cell lines), leading to an increase in NK-related studies.[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.[166]

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.

ACKNOWLEDGMENTS

We acknowledge the following support: NIH U01CA232488 (R.W., K.A.C., T.P.C., and D.A.L.); DOD NF170075 (T.P.C. and K.A.C.); Alex's Lemonade Stand Foundation (K.A.C.); NIH R01-CA222903 (K.A.C. and E.R.M.); CancerFree KIDS (M.G. and K.A.C.); NIH U54 CA232561 (T.P.C., E.R.M., D.A.L., K.A.C., M.S.C., and R.D.R.); NIH R21CA227926 (R.W.); NIH R01AI114581 (R.W.); and American Cancer Society 128436-RSG-15-180-01-LIB (R.W.).

REFERENCES

1. Yang, L., and Lin, P.C. (2017). Mechanisms that drive inflammatory tumor microenvironment, tumor heterogeneity, and metastatic progression. Semin. Cancer Biol. *47*, 185–195.
2. Radons, J. (2014). The role of inflammation in sarcoma. Adv. Exp. Med. Biol. *816*, 259–313.
3. Kohanbash, G., Carrera, D.A., Shrivastav, S., Ahn, B.J., Jahan, N., Mazor, T., Chheda, Z.S., Downey, K.M., Watchmaker, P.B., Beppler, C., et al. (2017). Isocitrate dehydrogenase mutations suppress STAT1 and CD8+ T cell accumulation in gliomas. J. Clin. Invest. *127*, 1425–1437.
4. Guo, X., Xue, H., Shao, Q., Wang, J., Guo, X., Chen, X., Zhang, J., Xu, S., Li, T., Zhang, P., et al. (2016). Hypoxia promotes glioma-associated macrophage infiltration via periostin and subsequent M2 polarization by upregulating TGF-beta and M-CSFR. Oncotarget *7*, 80521–80542.
5. Campbell, B.B., Light, N., Fabrizio, D., Zatzman, M., Fuligni, F., de Borja, R., Davidson, S., Edwards, M., Elvin, J.A., Hodel, K.P., et al. (2017). Comprehensive Analysis of Hypermutation in Human Cancer. Cell *171*, 1042–1056.e10.
6. Amayiri, N., Al-Hussaini, M., Swaidan, M., Jaradat, I., Qandeel, M., Tabori, U., Hawkins, C., Musharbash, A., Alsaad, K., and Bouffet, E. (2016). Synchronous glioblastoma and medulloblastoma in a child with mismatch repair mutation. Childs Nerv. Syst. *32*, 553–557.
7. Zhang, J., Walsh, M.F., Wu, G., Edmonson, M.N., Gruber, T.A., Easton, J., Hedges, D., Ma, X., Zhou, X., Yergeau, D.A., et al. (2015). Germline Mutations in Predisposition Genes in Pediatric Cancer. N. Engl. J. Med. *373*, 2336–2346.
8. Snell, L.M., McGaha, T.L., and Brooks, D.G. (2017). Type I Interferon in Chronic Virus Infection and Cancer. Trends Immunol. *38*, 542–557.
9. Duan, M., Li, W.C., Vlahos, R., Maxwell, M.J., Anderson, G.P., and Hibbs, M.L. (2012). Distinct macrophage subpopulations characterize acute infection and chronic inflammatory lung disease. J. Immunol. *189*, 946–955.
10. Kim, P.S., and Ahmed, R. (2010). Features of responding T cells in cancer and chronic infection. Curr. Opin. Immunol. *22*, 223–230.
11. Solinas, G., Germano, G., Mantovani, A., and Allavena, P. (2009). Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J. Leukoc. Biol. *86*, 1065–1073.
12. Mantovani, A., and Sica, A. (2010). Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr. Opin. Immunol. *22*, 231–237.
13. Doedens, A.L., Stockmann, C., Rubinstein, M.P., Liao, D., Zhang, N., Denardo, D.G., Coussens, L.M., Karin, M., Goldrath, A.W., and Johnson, R.S. (2010). Macrophage Expression of Hypoxia-Inducible Factor-1 alpha Suppresses T-Cell Function and Promotes Tumor Progression. Cancer Res. *70*, 7465–7475.
14. Majzner, R.G., Simon, J.S., Grosso, J.F., Martinez, D., Pawel, B.R., Santi, M., Merchant, M.S., Geoerger, B., Hezam, I., Marty, V., et al. (2017). Assessment of programmed death-ligand 1 expression and tumor-associated immune cells in pediatric cancer tissues. Cancer *123*, 3807–3815.
15. Koirala, P., Roth, M.E., Gill, J., Piperdi, S., Chinai, J.M., Geller, D.S., Hoang, B.H., Park, A., Fremed, M.A., Zang, X., and Gorlick, R. (2016). Immune infiltration and PD-L1 expression in the tumor microenvironment are prognostic in osteosarcoma. Sci. Rep. *6*, 30093.
16. Kumar, V., Patel, S., Tcyganov, E., and Gabrilovich, D.I. (2016). The Nature of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. Trends Immunol. *37*, 208–220.
17. Hao, N.B., Lü, M.H., Fan, Y.H., Cao, Y.L., Zhang, Z.R., and Yang, S.M. (2012). Macrophages in tumor microenvironments and the progression of tumors. Clin. Dev. Immunol. *2012*, 948098.
18. Spranger, S., Spaapen, R.M., Zha, Y., Williams, J., Meng, Y., Ha, T.T., and Gajewski, T.F. (2013). Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci. Transl. Med. *5*, 200ra116.
19. Wei, F., Zhong, S., Ma, Z., Kong, H., Medvec, A., Ahmed, R., Freeman, G.J., Krogsgaard, M., and Riley, J.L. (2013). Strength of PD-1 signaling differentially affects T-cell effector functions. Proc. Natl. Acad. Sci. USA *110*, E2480–E2489.
20. Monsurrò, V., Beghelli, S., Wang, R., Barbi, S., Coin, S., Di Pasquale, G., Bersani, S., Castellucci, M., Sorio, C., Eleuteri, S., et al. (2010). Anti-viral state segregates two molecular phenotypes of pancreatic adenocarcinoma: potential relevance for adenoviral gene therapy. J. Transl. Med. *8*, 10.
21. Duarte, C.W., Willey, C.D., Zhi, D., Cui, X., Harris, J.J., Vaughan, L.K., Mehta, T., McCubrey, R.O., Khodarev, N.N., Weichselbaum, R.R., and Gillespie, G.Y. (2012). Expression signature of IFN/STAT1 signaling genes predicts poor survival outcome in glioblastoma multiforme in a subtype-specific manner. PLoS ONE *7*, e29653.

22. Cosset, É., Petty, T.J., Dutoit, V., Cordey, S., Padioleau, I., Otten-Hernandez, P., Farinelli, L., Kaiser, L., Bruyère-Cerdan, P., Tirefort, D., et al. (2014). Comprehensive metagenomic analysis of glioblastoma reveals absence of known virus despite antiviral-like type I interferon gene response. Int. J. Cancer *135*, 1381–1389.

23. Laimer, K., Spizzo, G., Obrist, P., Gastl, G., Brunhuber, T., Schäfer, G., Norer, B., Rasse, M., Haffner, M.C., and Doppler, W. (2007). STAT1 activation in squamous cell cancer of the oral cavity: a potential predictive marker of response to adjuvant chemotherapy. Cancer *110*, 326–333.

24. Schultz, J., Koczan, D., Schmitz, U., Ibrahim, S.M., Pilch, D., Landsberg, J., and Kunz, M. (2010). Tumor-promoting role of signal transducer and activator of transcription (Stat)1 in late-stage melanoma growth. Clin. Exp. Metastasis *27*, 133–140.

25. Einav, U., Tabach, Y., Getz, G., Yitzhaky, A., Ozbek, U., Amariglio, N., Izraeli, S., Rechavi, G., and Domany, E. (2005). Gene expression analysis reveals a strong signature of an interferon-induced pathway in childhood lymphoblastic leukemia as well as in breast and ovarian cancer. Oncogene *24*, 6367–6375.

26. Ward, P.S., and Thompson, C.B. (2012). Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell *21*, 297–308.

27. Dang, C.V. (2013). MYC, metabolism, cell growth, and tumorigenesis. Cold Spring Harb. Perspect. Med. *3*, a014217.

28. Wang, T., Liu, G., and Wang, R. (2014). The Intercellular Metabolic Interplay between Tumor and Immune Cells. Front. Immunol. *5*, 358.

29. Pavlova, N.N., and Thompson, C.B. (2016). The Emerging Hallmarks of Cancer Metabolism. Cell Metab. *23*, 27–47.

30. O'Sullivan, D., and Pearce, E.L. (2015). Targeting T cell metabolism for therapy. Trends Immunol. *36*, 71–80.

31. DeNicola, G.M., and Cantley, L.C. (2015). Cancer's Fuel Choice: New Flavors for a Picky Eater. Mol. Cell *60*, 514–523.

32. Cairns, R.A., Harris, I.S., and Mak, T.W. (2011). Regulation of cancer cell metabolism. Nat. Rev. Cancer *11*, 85–95.

33. Hsu, P.P., and Sabatini, D.M. (2008). Cancer cell metabolism: Warburg and beyond. Cell *134*, 703–707.

34. Antonioli, L., Blandizzi, C., Pacher, P., and Haskó, G. (2013). Immunity, inflammation and cancer: a leading role for adenosine. Nat. Rev. Cancer *13*, 842–857.

35. Antonioli, L., Pacher, P., Vizi, E.S., and Haskó, G. (2013). CD39 and CD73 in immunity and inflammation. Trends Mol. Med. *19*, 355–367.

36. Kumar, V. (2013). Adenosine as an endogenous immunoregulator in cancer pathogenesis: where to go? Purinergic Signal. *9*, 145–165.

37. Stagg, J., and Smyth, M.J. (2010). Extracellular adenosine triphosphate and adenosine in cancer. Oncogene *29*, 5346–5358.

38. Allard, D., Allard, B., Gaudreau, P.O., Chrobak, P., and Stagg, J. (2016). CD73-adenosine: a next-generation target in immuno-oncology. Immunotherapy *8*, 145–163.

39. Ghalamfarsa, G., Kazemi, M.H., Raoofi Mohseni, S., Masjedi, A., Hojjat-Farsangi, M., Azizi, G., Yousefi, M., and Jadidi-Niaragh, F. (2019). CD73 as a potential opportunity for cancer immunotherapy. Expert Opin. Ther. Targets *23*, 127–142.

40. Dupain, C., Harttrampf, A.C., Urbinati, G., Geoerger, B., and Massaad-Massade, L. (2017). Relevance of Fusion Genes in Pediatric Cancers: Toward Precision Medicine. Mol. Ther. Nucleic Acids *6*, 315–326.

41. Turc-Carel, C., Aurias, A., Mugneret, F., Lizard, S., Sidaner, I., Volk, C., Thiery, J.P., Olschwang, S., Philip, I., Berger, M.P., et al. (1988). Chromosomes in Ewing's sarcoma. I. An evaluation of 85 cases of remarkable consistency of t(11;22)(q24;q12). Cancer Genet. Cytogenet. *32*, 229–238.

42. May, W.A., Lessnick, S.L., Braun, B.S., Klemsz, M., Lewis, B.C., Lunsford, L.B., Hromas, R., and Denny, C.T. (1993). The Ewing's sarcoma EWS/FLI-1 fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than FLI-1. Mol. Cell. Biol. *13*, 7393–7398.

43. Feng, F.Y., Brenner, J.C., Hussain, M., and Chinnaiyan, A.M. (2014). Molecular pathways: targeting ETS gene fusions in cancer. Clin. Cancer Res. *20*, 4442–4448.

44. Ohno, T., Rao, V.N., and Reddy, E.S. (1993). EWS/Fli-1 chimeric protein is a transcriptional activator. Cancer Res. *53*, 5859–5863.

45. Grohar, P.J., Segars, L.E., Yeung, C., Pommier, Y., D'Incalci, M., Mendoza, A., and Helman, L.J. (2014). Dual targeting of EWS-FLI1 activity and the associated DNA damage response with trabectedin and SN38 synergistically inhibits Ewing sarcoma cell growth. Clin. Cancer Res. *20*, 1190–1203.

46. Jacques, C., Lamoureux, F., Baud'huin, M., Rodriguez Calleja, L., Quillard, T., Amiaud, J., Tirode, F., Rédini, F., Bradner, J.E., Heymann, D., and Ory, B. (2016). Targeting the epigenetic readers in Ewing sarcoma inhibits the oncogenic transcription factor EWS/Fli1. Oncotarget *7*, 24125–24140.

47. Pishas, K.I., and Lessnick, S.L. (2016). Recent advances in targeted therapy for Ewing sarcoma. F1000Res. *5*, F1000.

48. Theisen, E.R., Pishas, K.I., Saund, R.S., and Lessnick, S.L. (2016). Therapeutic opportunities in Ewing sarcoma: EWS-FLI inhibition via LSD1 targeting. Oncotarget *7*, 17616–17630.

49. Sorensen, P.H., Lynch, J.C., Qualman, S.J., Tirabosco, R., Lim, J.F., Maurer, H.M., Bridge, J.A., Crist, W.M., Triche, T.J., and Barr, F.G. (2002). PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the children's oncology group. J. Clin. Oncol. *20*, 2672–2679.

50. Davis, R.J., D'Cruz, C.M., Lovell, M.A., Biegel, J.A., and Barr, F.G. (1994). Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. Cancer Res. *54*, 2869–2872.

51. Barr, F.G. (2001). Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma. Oncogene *20*, 5736–5746.

52. Davidoff, A.M. (2010). Pediatric oncology. Semin. Pediatr. Surg. *19*, 225–233.

53. Marshall, A.D., and Grosveld, G.C. (2012). Alveolar rhabdomyosarcoma - The molecular drivers of PAX3/7-FOXO1-induced tumorigenesis. Skelet. Muscle *2*, 25.

54. Li, Y., Wang, L.X., Pang, P., Twitty, C., Fox, B.A., Aung, S., Urba, W.J., and Hu, H.M. (2009). Cross-presentation of tumor associated antigens through tumor-derived autophagosomes. Autophagy *5*, 576–577.

55. Marshall, G.M., Carter, D.R., Cheung, B.B., Liu, T., Mateos, M.K., Meyerowitz, J.G., and Weiss, W.A. (2014). The prenatal origins of cancer. Nat. Rev. Cancer *14*, 277–289.

56. Cheever, M.A., Allison, J.P., Ferris, A.S., Finn, O.J., Hastings, B.M., Hecht, T.T., Mellman, I., Prindiville, S.A., Viner, J.L., Weiner, L.M., and Matrisian, L.M. (2009). The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin. Cancer Res. *15*, 5323–5337.

57. Liu, X.S., and Mardis, E.R. (2017). Applications of Immunogenomics to Cancer. Cell *168*, 600–612.

58. Gros, A., Parkhurst, M.R., Tran, E., Pasetto, A., Robbins, P.F., Ilyas, S., Prickett, T.D., Gartner, J.J., Crystal, J.S., Roberts, I.M., et al. (2016). Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. Nat. Med. *22*, 433–438.

59. Ghonime, M.G., Jackson, J., Shah, A., Roth, J., Li, M., Saunders, U., Coleman, J., Gillespie, G.Y., Markert, J.M., and Cassady, K.A. (2018). Chimeric HCMV/HSV-1 and $\Delta\gamma_1$34.5 oncolytic herpes simplex virus elicit immune mediated antigliomal effect and antitumor memory. Transl. Oncol. *11*, 86–93.

60. Ghonime, M.G., and Cassady, K.A. (2018). Combination Therapy Using Ruxolitinib and Oncolytic HSV Renders Resistant MPNSTs Susceptible to Virotherapy. Cancer Immunol. Res. *6*, 1499–1510.

61. Steed, A.L., and Stappenbeck, T.S. (2014). Role of viruses and bacteria-virus interactions in autoimmunity. Curr. Opin. Immunol. *31*, 102–107.

62. Getts, D.R., Chastain, E.M., Terry, R.L., and Miller, S.D. (2013). Virus infection, antiviral immunity, and autoimmunity. Immunol. Rev. *255*, 197–209.

63. Harley, J.B., and James, J.A. (2006). Epstein-Barr virus infection induces lupus autoimmunity. Bull. NYU Hosp. Jt. Dis. *64*, 45–50.

64. McCoy, L., Tsunoda, I., and Fujinami, R.S. (2006). Multiple sclerosis and virus induced immune responses: autoimmunity can be primed by molecular mimicry and augmented by bystander activation. Autoimmunity *39*, 9–19.

65. Olson, J.K., Croxford, J.L., and Miller, S.D. (2001). Virus-induced autoimmunity: potential role of viruses in initiation, perpetuation, and progression of T-cell-mediated autoimmune disease. Viral Immunol. *14*, 227–250.

66. Nauclér, C.S., Larsson, S., and Möller, E. (1996). A novel mechanism for virus-induced autoimmunity in humans. Immunol. Rev. *152*, 175–192.

67. Griffin, D.E. (1976). Role of the immune response in age-dependent resistance of mice to encephalitis due to Sindbis virus. J. Infect. Dis. *133*, 456–464.

68. Griffin, D.E. (2010). Recovery from viral encephalomyelitis: immune-mediated noncytolytic virus clearance from neurons. Immunol. Res. *47*, 123–133.

69. Johnson, R.T., McFarland, H.F., and Levy, S.E. (1972). Age-dependent resistance to viral encephalitis: studies of infections due to Sindbis virus in mice. J. Infect. Dis. *125*, 257–262.

70. Labrada, L., Liang, X.H., Zheng, W., Johnston, C., and Levine, B. (2002). Age-dependent resistance to lethal alphavirus encephalitis in mice: analysis of gene expression in the central nervous system and identification of a novel interferon-inducible protective gene, mouse ISG12. J. Virol. *76*, 11688–11703.

71. Taylor, G.S., Long, H.M., Brooks, J.M., Rickinson, A.B., and Hislop, A.D. (2015). The immunology of Epstein-Barr virus-induced disease. Annu. Rev. Immunol. *33*, 787–821.

72. Harik, N., and DeBiasi, R.L. (2018). Neonatal nonpolio enterovirus and parechovirus infections. Semin. Perinatol. *42*, 191–197.

73. Faden, H., Wynn, R.J., Campagna, L., and Ryan, R.M. (2005). Outbreak of adenovirus type 30 in a neonatal intensive care unit. J. Pediatr. *146*, 523–527.

74. Ronchi, A., Doern, C., Brock, E., Pugni, L., and Sánchez, P.J. (2014). Neonatal adenoviral infection: a seventeen year experience and review of the literature. J. Pediatr. *164*, 529–535.e1–4.

75. Abzug, M.J., and Levin, M.J. (1991). Neonatal adenovirus infection: four patients and review of the literature. Pediatrics *87*, 890–896.

76. McJunkin, J.E., de los Reyes, E.C., Irazuzta, J.E., Caceres, M.J., Khan, R.R., Minnich, L.L., Fu, K.D., Lovett, G.D., Tsai, T., and Thompson, A. (2001). La Crosse encephalitis in children. N. Engl. J. Med. *344*, 801–807.

77. Maccario, R., Notarangelo, L.D., Montagna, D., Vitiello, A., Porta, F.A., Lanfranchi, A., Marseglia, G., and Ugazio, A.G. (1986). Lymphocyte subpopulations in the neonate: in vitro proliferation, IL-1 and IL-2 production by a subset of HNK-1-, OKT3-, OKT8+ lymphocytes displaying NK activity. Thymus *8*, 225–234.

78. Prendergast, A.J., Klenerman, P., and Goulder, P.J. (2012). The impact of differential antiviral immunity in children and adults. Nat. Rev. Immunol. *12*, 636–648.

79. Wilson, C.B. (1986). Immunologic basis for increased susceptibility of the neonate to infection. J. Pediatr. *108*, 1–12.

80. Quie, P.G. (1990). Antimicrobial defenses in the neonate. Semin. Perinatol. *14*, 2–9.

81. Clerici, M., DePalma, L., Roilides, E., Baker, R., and Shearer, G.M. (1993). Analysis of T helper and antigen-presenting cell functions in cord blood and peripheral blood leukocytes from healthy children of different ages. J. Clin. Invest. *91*, 2829–2836.

82. Lewinsohn, D.A., Gennaro, M.L., Scholvinck, L., and Lewinsohn, D.M. (2004). Tuberculosis immunology in children: diagnostic and therapeutic challenges and opportunities. Int. J. Tuberc. Lung Dis. *8*, 658–674.

83. Kumagai, T., Nagai, K., Okui, T., Tsutsumi, H., Nagata, N., Yano, S., Nakayama, T., Okuno, Y., and Kamiya, H. (2004). Poor immune responses to influenza vaccination in infants. Vaccine *22*, 3404–3410.

84. Roilides, E., Clerici, M., Depalma, L., Baker, R., Pizzo, P.A., and Shearer, G.M. (1991). Differential Maturation of T-Helper Cell-Function in Neonates and Young-Children. FASEB J. *5*, A1699.

85. Verhoeven, D., Perry, S., and Pryharski, K. (2016). Control of influenza infection is impaired by diminished interferon-γ secretion by CD4 T cells in the lungs of toddler mice. J. Leukoc. Biol. *100*, 203–212.

86. Brint, M.E., Hughes, J.M., Shah, A., Miller, C.R., Harrison, L.G., Meals, E.A., Blanch, J., Thompson, C.R., Cormier, S.A., and DeVincenzo, J.P. (2017). Prolonged viral replication and longitudinal viral dynamic differences among respiratory syncytial virus infected infants. Pediatr. Res. *82*, 872–880.

87. Li, I.W., Hung, I.F., To, K.K., Chan, K.H., Wong, S.S., Chan, J.F., Cheng, V.C., Tsang, O.T., Lai, S.T., Lau, Y.L., and Yuen, K.Y. (2010). The natural viral load profile of patients with pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment. Chest *137*, 759–768.

88. Redlberger-Fritz, M., Hirk, S., Buchinger, D., Haberl, R., Hell, M., Perkmann-Nagele, N., Kundi, M., and Popow-Kraupp, T. (2014). Distinct differences in clinical manifestation and viral laboratory parameters between children and adults with influenza A(H1N1)pdm09 infection–a retrospective comparative analysis. J. Med. Virol. *86*, 1048–1055.

89. Streby, K.A., Geller, J.I., Currier, M.A., Warren, P.S., Racadio, J.M., Towbin, A.J., Vaughan, M.R., Triplet, M., Ott-Napier, K., Dishman, D.J., et al. (2017). Intratumoral Injection of HSV1716, an Oncolytic Herpes Virus, Is Safe and Shows Evidence of Immune Response and Viral Replication in Young Cancer Patients. Clin. Cancer Res. *23*, 3566–3574.

90. Chen, C.Y., Wang, P.Y., Hutzen, B., Sprague, L., Swain, H.M., Love, J.K., Stanek, J.R., Boon, L., Conner, J., and Cripe, T.P. (2017). Cooperation of Oncolytic Herpes Virotherapy and PD-1 Blockade in Murine Rhabdomyosarcoma Models. Sci. Rep. *7*, 2396.

91. Hammill, A.M., Conner, J., and Cripe, T.P. (2010). Oncolytic virotherapy reaches adolescence. Pediatr. Blood Cancer *55*, 1253–1263.

92. Smith, E.R., and Chiocca, E.A. (2000). Oncolytic viruses as novel anticancer agents: turning one scourge against another. Expert Opin. Investig. Drugs *9*, 311–327.

93. Varghese, S., and Rabkin, S.D. (2002). Oncolytic herpes simplex virus vectors for cancer virotherapy. Cancer Gene Ther. *9*, 967–978.

94. Arceci, R.J., and Cripe, T.P. (2002). Emerging cancer-targeted therapies. Pediatr. Clin. North Am. *49*, 1339–1368, vii–viii.

95. Walker, J.R., McGeagh, K.G., Sundaresan, P., Jorgensen, T.J., Rabkin, S.D., and Martuza, R.L. (1999). Local and systemic therapy of human prostate adenocarcinoma with the conditionally replicating herpes simplex virus vector G207. Hum. Gene Ther. *10*, 2237–2243.

96. Oyama, M., Ohigashi, T., Hoshi, M., Nakashima, J., Tachibana, M., Murai, M., Uyemura, K., and Yazaki, T. (2000). Intravesical and intravenous therapy of human bladder cancer by the herpes vector G207. Hum. Gene Ther. *11*, 1683–1693.

97. Nakamori, M., Fu, X., Pettaway, C.A., and Zhang, X. (2004). Potent antitumor activity after systemic delivery of a doubly fusogenic oncolytic herpes simplex virus against metastatic prostate cancer. Prostate *60*, 53–60.

98. Wong, R.J., Chan, M.-K., Yu, Z., Kim, T.H., Bhargava, A., Stiles, B.M., Horsburgh, B.C., Shah, J.P., Ghossein, R.A., Singh, B., and Fong, Y. (2004). Effective intravenous therapy of murine pulmonary metastases with an oncolytic herpes virus expressing interleukin 12. Clin. Cancer Res. *10*, 251–259.

99. Varghese, S., Rabkin, S.D., Nielsen, G.P., MacGarvey, U., Liu, R., and Martuza, R.L. (2007). Systemic therapy of spontaneous prostate cancer in transgenic mice with oncolytic herpes simplex viruses. Cancer Res. *67*, 9371–9379.

100. Veerapong, J., Bickenbach, K.A., Shao, M.Y., Smith, K.D., Posner, M.C., Roizman, B., and Weichselbaum, R.R. (2007). Systemic delivery of (gamma1)34.5-deleted herpes simplex virus-1 selectively targets and treats distant human xenograft tumors that express high MEK activity. Cancer Res. *67*, 8301–8306.

101. Conner, J., Braidwood, L., and Brown, S.M. (2008). A strategy for systemic delivery of the oncolytic herpes virus HSV1716: redirected tropism by antibody-binding sites incorporated on the virion surface as a glycoprotein D fusion protein. Gene Ther. *15*, 1579–1592.

102. Eshun, F.K., Currier, M.A., Gillespie, R.A., Fitzpatrick, J.L., Baird, W.H., and Cripe, T.P. (2010). VEGF blockade decreases the tumor uptake of systemic oncolytic herpes virus but enhances therapeutic efficacy when given after virotherapy. Gene Ther. *17*, 922–929.

103. Cassady, K.A., Haworth, K.B., Jackson, J., Markert, J.M., and Cripe, T.P. (2016). To Infection and Beyond: The Multi-Pronged Anti-Cancer Mechanisms of Oncolytic Viruses. Viruses *8*, E43.

104. Friedman, G.K., Nan, L., Haas, M.C., Kelly, V.M., Moore, B.P., Langford, C.P., Xu, H., Han, X., Beierle, E.A., Markert, J.M., et al. (2015). $\gamma_1$34.5-deleted HSV-1-expressing human cytomegalovirus IRS1 gene kills human glioblastoma cells as efficiently as wild-type HSV-1 in normoxia or hypoxia. Gene Ther. *22*, 356.

105. Wang, P.Y., Swain, H.M., Kunkler, A.L., Chen, C.Y., Hutzen, B.J., Arnold, M.A., Streby, K.A., Collins, M.H., Dipasquale, B., Stanek, J.R., et al. (2016). Neuroblastomas vary widely in their sensitivities to herpes simplex virotherapy unrelated to virus receptors and susceptibility. Gene Ther. *23*, 135–143.

106. Jackson, J.D., Markert, J.M., Li, L., Carroll, S.L., and Cassady, K.A. (2016). STAT1 and NF-κB Inhibitors Diminish Basal Interferon-Stimulated Gene Expression and Improve the Productive Infection of Oncolytic HSV in MPNST Cells. Mol. Cancer Res. *14*, 482–492.

107. Jackson, J.D., McMorris, A.M., Roth, J.C., Coleman, J.M., Whitley, R.J., Gillespie, G.Y., Carroll, S.L., Markert, J.M., and Cassady, K.A. (2014). Assessment of oncolytic HSV efficacy following increased entry-receptor expression in malignant peripheral nerve sheath tumor cell lines. Gene Ther. *21*, 984–990.

108. Hellums, E.K., Markert, J.M., Parker, J.N., He, B., Perbal, B., Roizman, B., Whitley, R.J., Langford, C.P., Bharara, S., and Gillespie, G.Y. (2005). Increased efficacy of an interleukin-12-secreting herpes simplex virus in a syngeneic intracranial murine glioma model. Neuro-oncol. *7*, 213–224.

109. Okada, H., and Pollack, I.F. (2004). Cytokine gene therapy for malignant glioma. Expert Opin. Biol. Ther. *4*, 1609–1620.

110. Markert, J.M., Medlock, M.D., Rabkin, S.D., Gillespie, G.Y., Todo, T., Hunter, W.D., Palmer, C.A., Feigenbaum, F., Tornatore, C., Tufaro, F., and Martuza, R.L. (2000). Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther. *7*, 867–874.

111. Parker, J.N., Gillespie, G.Y., Love, C.E., Randall, S., Whitley, R.J., and Markert, J.M. (2000). Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. Proc. Natl. Acad. Sci. USA *97*, 2208–2213.

112. Andreansky, S., He, B., van Cott, J., McGhee, J., Markert, J.M., Gillespie, G.Y., Roizman, B., and Whitley, R.J. (1998). Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. Gene Ther. *5*, 121–130.

113. Chambers, R., Gillespie, G.Y., Soroceanu, L., Andreansky, S., Chatterjee, S., Chou, J., Roizman, B., and Whitley, R.J. (1995). Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a scid mouse model of human malignant glioma. Proc. Natl. Acad. Sci. USA *92*, 1411–1415.

114. Shah, A.C., Parker, J.N., Gillespie, G.Y., Lakeman, F.D., Meleth, S., Markert, J.M., and Cassady, K.A. (2007). Enhanced antiglioma activity of chimeric HCMV/HSV-1 oncolytic viruses. Gene Ther. *14*, 1045–1054.

115. Cassady, K.A., Bauer, D.F., Roth, J., Chambers, M.R., Shoeb, T., Coleman, J., Prichard, M., Gillespie, G.Y., and Markert, J.M. (2017). Pre-clinical Assessment of C134, a Chimeric Oncolytic Herpes Simplex Virus, in Mice and Non-human Primates. Mol. Ther. Oncolytics *5*, 1–10.

116. Cassady, K.A., Saunders, U., and Shimamura, M. (2012). $\Delta\gamma_1$134.5 herpes simplex viruses encoding human cytomegalovirus IRS1 or TRS1 induce interferon regulatory factor 3 phosphorylation and an interferon-stimulated gene response. J. Virol. *86*, 610–614.

117. Cassady, K.A. (2005). Human cytomegalovirus TRS1 and IRS1 gene products block the double-stranded-RNA-activated host protein shutoff response induced by herpes simplex virus type 1 infection. J. Virol. *79*, 8707–8715.

118. Hutzen, B., Chen, C.Y., Wang, P.Y., Sprague, L., Swain, H.M., Love, J., Conner, J., Boon, L., and Cripe, T.P. (2017). TGF-β Inhibition Improves Oncolytic Herpes Viroimmunotherapy in Murine Models of Rhabdomyosarcoma. Mol. Ther. Oncolytics *7*, 17–26.

119. Chen, C.Y., Hutzen, B., Wedekind, M.F., and Cripe, T.P. (2018). Oncolytic virus and PD-1/PD-L1 blockade combination therapy. Oncolytic Virother. *7*, 65–77.

120. Zamarin, D., and Wolchok, J.D. (2014). Potentiation of immunomodulatory antibody therapy with oncolytic viruses for treatment of cancer. Mol. Ther. Oncolytics *1*, 14004.

121. Pardoll, D.M. (2012). The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer *12*, 252–264.

122. Turtle, C.J. (2014). Chimeric antigen receptor modified T cell therapy for B cell malignancies. Int. J. Hematol. *99*, 132–140.

123. Enblad, G., Karlsson, H., Gammelgård, G., Wenthe, J., Lövgren, T., Amini, R.M., Wikstrom, K.I., Essand, M., Savoldo, B., Hallböök, H., et al. (2018). A Phase I/IIa Trial Using CD19-Targeted Third-Generation CAR T Cells for Lymphoma and Leukemia. Clin. Cancer Res. *24*, 6185–6194.

124. Zhang, H., and Chen, J. (2018). Current status and future directions of cancer immunotherapy. J. Cancer *9*, 1773–1781.

125. Xin, G., Schauder, D.M., Jing, W., Jiang, A., Joshi, N.S., Johnson, B., and Cui, W. (2017). Pathogen boosted adoptive cell transfer immunotherapy to treat solid tumors. Proc. Natl. Acad. Sci. USA *114*, 740–745.

126. Menon, S., Shin, S., and Dy, G. (2016). Advances in Cancer Immunotherapy in Solid Tumors. Cancers (Basel) *8*, E106.

127. Gardner, R., Wu, D., Cherian, S., Fang, M., Hanafi, L.A., Finney, O., Smithers, H., Jensen, M.C., Riddell, S.R., Maloney, D.G., and Turtle, C.J. (2016). Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood *127*, 2406–2410.

128. DeSelm, C., Palomba, M.L., Yahalom, J., Hamieh, M., Eyquem, J., Rajasekhar, V.K., and Sadelain, M. (2018). Low-Dose Radiation Conditioning Enables CAR T Cells to Mitigate Antigen Escape. Mol. Ther. *26*, 2542–2552.

129. Grupp, S.A., Kalos, M., Barrett, D., Aplenc, R., Porter, D.L., Rheingold, S.R., Teachey, D.T., Chew, A., Hauck, B., Wright, J.F., et al. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N. Engl. J. Med. *368*, 1509–1518.

130. Maude, S.L., Frey, N., Shaw, P.A., Aplenc, R., Barrett, D.M., Bunin, N.J., Chew, A., Gonzalez, V.E., Zheng, Z., Lacey, S.F., et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. N. Engl. J. Med. *371*, 1507–1517.

131. Lee, D.W., Barrett, D.M., Mackall, C., Orentas, R., and Grupp, S.A. (2012). The future is now: chimeric antigen receptors as new targeted therapies for childhood cancer. Clin. Cancer Res. *18*, 2780–2790.

132. Campana, D., Schwarz, H., and Imai, C. (2014). 4-1BB chimeric antigen receptors. Cancer J. *20*, 134–140.

133. Porter, D.L., Levine, B.L., Kalos, M., Bagg, A., and June, C.H. (2011). Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N. Engl. J. Med. *365*, 725–733.

134. Lee, D.W., Gardner, R., Porter, D.L., Louis, C.U., Ahmed, N., Jensen, M., Grupp, S.A., and Mackall, C.L. (2014). Current concepts in the diagnosis and management of cytokine release syndrome. Blood *124*, 188–195.

135. Gonzalez Castro, L.N., Dietrich, J., and Forst, D.A. (2019). Stuttering as the first sign of CAR-T-cell-related encephalopathy syndrome (CRES). J. Cancer Res. Clin. Oncol. *145*, 1917–1918.

136. Freud, A.G., Mundy-Bosse, B.L., Yu, J., and Caligiuri, M.A. (2017). The Broad Spectrum of Human Natural Killer Cell Diversity. Immunity *47*, 820–833.

137. Gras Navarro, A., Björklund, A.T., and Chekenya, M. (2015). Therapeutic potential and challenges of natural killer cells in treatment of solid tumors. Front. Immunol. *6*, 202.

138. Romee, R., Cooley, S., Berrien-Elliott, M.M., Westervelt, P., Verneris, M.R., Wagner, J.E., Weisdorf, D.J., Blazar, B.R., Ustun, C., DeFor, T.E., et al. (2018). First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation. Blood *131*, 2515–2527.

139. Guillerey, C., Huntington, N.D., and Smyth, M.J. (2016). Targeting natural killer cells in cancer immunotherapy. Nat. Immunol. *17*, 1025–1036.

140. Bradley, M., Zeytun, A., Rafi-Janajreh, A., Nagarkatti, P.S., and Nagarkatti, M. (1998). Role of spontaneous and interleukin-2-induced natural killer cell activity in the cytotoxicity and rejection of Fas+ and Fas- tumor cells. Blood *92*, 4248–4255.

141. Kayagaki, N., Yamaguchi, N., Nakayama, M., Takeda, K., Akiba, H., Tsutsui, H., Okamura, H., Nakanishi, K., Okumura, K., and Yagita, H. (1999). Expression and function of TNF-related apoptosis-inducing ligand on murine activated NK cells. J. Immunol. *163*, 1906–1913.

142. Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W.D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., et al. (2002). Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science *295*, 2097–2100.

143. Dunbar, E.M., Buzzeo, M.P., Levine, J.B., Schold, J.D., Meier-Kriesche, H.U., and Reddy, V. (2008). The relationship between circulating natural killer cells after reduced intensity conditioning hematopoietic stem cell transplantation and relapse-free survival and graft-versus-host disease. Haematologica *93*, 1852–1858.

144. Savani, B.N., Mielke, S., Adams, S., Uribe, M., Rezvani, K., Yong, A.S., Zeilah, J., Kurlander, R., Srinivasan, R., Childs, R., et al. (2007). Rapid natural killer cell recovery determines outcome after T-cell-depleted HLA-identical stem cell

transplantation in patients with myeloid leukemias but not with acute lymphoblastic leukemia. Leukemia *21*, 2145–2152.

145. Denman, C.J., Senyukov, V.V., Somanchi, S.S., Phatarpekar, P.V., Kopp, L.M., Johnson, J.L., Singh, H., Hurton, L., Maiti, S.N., Huls, M.H., et al. (2012). Membrane-bound IL-21 promotes sustained ex vivo proliferation of human natural killer cells. PLoS ONE *7*, e30264.

146. Somanchi, S.S., and Lee, D.A. (2016). Ex Vivo Expansion of Human NK Cells Using K562 Engineered to Express Membrane Bound IL21. Methods Mol. Biol. *1441*, 175–193.

147. Somanchi, S.S., Senyukov, V.V., Denman, C.J., and Lee, D.A. (2011). Expansion, purification, and functional assessment of human peripheral blood NK cells. J. Vis. Exp. (48), 2540.

148. Kantoff, P.W., Higano, C.S., Shore, N.D., Berger, E.R., Small, E.J., Penson, D.F., Redfern, C.H., Ferrari, A.C., Dreicer, R., Sims, R.B., et al.; IMPACT Study Investigators (2010). Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N. Engl. J. Med. *363*, 411–422.

149. Ardon, H., De Vleeschouwer, S., Van Calenbergh, F., Claes, L., Kramm, C.M., Rutkowski, S., Wolff, J.E., and Van Gool, S.W. (2010). Adjuvant dendritic cell-based tumour vaccination for children with malignant brain tumours. Pediatr. Blood Cancer *54*, 519–525.

150. Caruso, D.A., Orme, L.M., Neale, A.M., Radcliff, F.J., Amor, G.M., Maixner, W., Downie, P., Hassall, T.E., Tang, M.L., and Ashley, D.M. (2004). Results of a phase 1 study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. Neuro-oncol. *6*, 236–246.

151. Nestle, F.O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G., and Schadendorf, D. (1998). Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat. Med. *4*, 328–332.

152. Thurner, B., Haendle, I., Röder, C., Dieckmann, D., Keikavoussi, P., Jonuleit, H., Bender, A., Maczek, C., Schreiner, D., von den Driesch, P., et al. (1999). Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J. Exp. Med. *190*, 1669–1678.

153. Lim, S.H., and Bailey-Wood, R. (1999). Idiotypic protein-pulsed dendritic cell vaccination in multiple myeloma. Int. J. Cancer *83*, 215–222.

154. Geiger, J.D., Hutchinson, R.J., Hohenkirk, L.F., McKenna, E.A., Yanik, G.A., Levine, J.E., Chang, A.E., Braun, T.M., and Mulé, J.J. (2001). Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res. *61*, 8513–8519.

155. Elster, J.D., Krishnadas, D.K., and Lucas, K.G. (2016). Dendritic cell vaccines: A review of recent developments and their potential pediatric application. Hum. Vaccin. Immunother. *12*, 2232–2239.

156. Kolb, E.A., Sampson, V., Stabley, D., Walter, A., Sol-Church, K., Cripe, T., Hingorani, P., Ahern, C.H., Weigel, B.J., Zwiebel, J., and Blaney, S.M. (2015). A phase I trial and viral clearance study of reovirus (Reolysin) in children with relapsed or refractory extra-cranial solid tumors: a Children's Oncology Group Phase I Consortium report. Pediatr. Blood Cancer *62*, 751–758.

157. Waters, A.M., Friedman, G.K., Ring, E.K., and Beierle, E.A. (2016). Oncolytic virotherapy for pediatric malignancies: future prospects. Oncolytic Virother. *5*, 73–80.

158. Burke, M.J., Ahern, C., Weigel, B.J., Poirier, J.T., Rudin, C.M., Chen, Y., Cripe, T.P., Bernhardt, M.B., and Blaney, S.M. (2015). Phase I trial of Seneca Valley Virus (NTX-010) in children with relapsed/refractory solid tumors: a report of the Children's Oncology Group. Pediatr. Blood Cancer *62*, 743–750.

159. Cripe, T.P., Ngo, M.C., Geller, J.I., Louis, C.U., Currier, M.A., Racadio, J.M., Towbin, A.J., Rooney, C.M., Pelusio, A., Moon, A., et al. (2015). Phase 1 study of intratumoral Pexa-Vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus, in pediatric cancer patients. Mol. Ther. *23*, 602–608.

160. Waters, A.M., Johnston, J.M., Reddy, A.T., Fiveash, J., Madan-Swain, A., Kachurak, K., Bag, A.K., Gillespie, G.Y., Markert, J.M., and Friedman, G.K. (2017). Rationale and Design of a Phase I Clinical Trial to Evaluate HSV G207 Alone or with a Single Radiation Dose in Children with Progressive or Recurrent Malignant Supratentorial Brain Tumors. Hum. Gene Ther. Clin. Dev. , Published online February 24, 2017. https://doi.org/10.1089/hum.2017.002.

161. Sheridan, C. (2013). Amgen announces oncolytic virus shrinks tumors. Nat. Biotechnol. *31*, 471–472.

162. Orr, M.T., Edelmann, K.H., Vieira, J., Corey, L., Raulet, D.H., and Wilson, C.B. (2005). Inhibition of MHC class I is a virulence factor in herpes simplex virus infection of mice. PLoS Pathog. *1*, e7.

163. Mohr, I., and Gluzman, Y. (1996). A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. EMBO J. *15*, 4759–4766.

164. Cassady, K.A., Gross, M., and Roizman, B. (1998). The herpes simplex virus US11 protein effectively compensates for the gamma1(34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the alpha subunit of eukaryotic translation initiation factor 2. J. Virol. *72*, 8620–8626.

165. Cassady, K.A., Gross, M., and Roizman, B. (1998). The second-site mutation in the herpes simplex virus recombinants lacking the gamma134.5 genes precludes shutoff of protein synthesis by blocking the phosphorylation of eIF-2alpha. J. Virol. *72*, 7005–7011.

166. Krishnadas, D.K., Shusterman, S., Bai, F., Diller, L., Sullivan, J.E., Cheerva, A.C., George, R.E., and Lucas, K.G. (2015). A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. Cancer Immunol. Immunother. *64*, 1251–1260.