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REVIEW

Dendritic cell vaccines: A review of recent developments and their potential pediatric application

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ABSTRACT

For many cancers the use of conventional chemotherapy has been maximized, and further intensification of chemotherapy generally results in excess toxicity with little long-term benefit for cure. Many tumors become resistant to chemotherapy, making the investigation of novel approaches such as immunotherapy of interest. Because the tumor microenvironment is known to promote immune tolerance and down regulate the body's natural defense mechanisms, modulating the immune system with the use of dendritic cell (DC) therapy is an attractive approach. Thousands of patients with diverse tumor types have been treated with DC vaccines. While antigen specific immune responses have been reported, the duration and magnitude of these responses are typically weak, and objective clinical responses have been limited. DC vaccine generation and administration is a multi-step process with opportunities for improvement in source of DC for vaccine, selection of target antigen, and boosting effector cell response via administration of vaccine adjuvant or concomitant pharmacologic immunomodulation. In this review we will discuss recent developments in each of these areas and highlight elements that could be moved into pediatric clinical trials.

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Approximately 15,780 children age 0–19 y are diagnosed with cancer in the US each year.[1] While improved surgical, chemotherapeutic, and radiation based approaches have increasingly resulted in cure for many of these diseases, almost 2000 children die of cancer every year in the US and current treatment regimens are associated with significant toxicity. In addition, children with relapsed cancer often have limited curative options, making development of immune-based strategies of interest. Further improvements in cure rate are likely to come from adjuvant therapies. Because the tumor microenvironment is known in many cases to promote immune tolerance, one attractive approach is modulating the immune system to stimulate antitumor immune response.

Dendritic Cells (DC) are the most powerful antigen presenting cells of the immune system, capable of stimulating naïve and memory $CD8^+$ T-cells as well as B-cells and $CD4^+$ helper T-cells. In the immature state DC are present in blood and tissues, processing foreign antigens for presentation to the immune system. The uptake of presentable antigen stimulates maturation of DC and promotes DC migration to lymph nodes, where these cells can directly interact with immune effector cells. Mature DC are capable of stimulating T helper type-1 immune responses and antigen specific $CD8^+$ cytotoxic T-lymphocytes (CTL), but within the tumor microenvironment DC promote tumor tolerance, facilitating T helper type-2 responses. Therefore DC can exert both strong positive and negative influences on the acquisition of tumor specific cellular immune responses.

DC vaccines have generally consisted of autologous monocytes that are matured *in vitro* and pulsed with antigen before injection. These vaccines have been given to thousands of patients of all ages with diverse tumor types and have been generally well-tolerated with little toxicity beyond local skin reactions.[2,3] While antigen specific immune responses have been reported in a number of these trials, the duration and magnitude of these responses are typically weak, and objective clinical responses have been limited. Sipuleucel-T, an autologous dendritic cell vaccine primed with a recombinant antigen composed of prostatic acid phosphatase linked to GM-CSF as an adjuvant, is the only DC vaccine which has shown sufficient efficacy in a Phase III clinical trial to gain FDA approval.[4] While this vaccine is targeted to an adult malignancy, its success does offer hope that an effective DC vaccine can be developed for pediatric tumors. Clinical responses to DC vaccines in children with malignant solid tumors have been disappointing to date, with excellent tolerability but poor efficacy both in high grade CNS tumors[5-7] and in a more diverse group of recurrent solid tumors.[3,8-14]

Each step of DC vaccine production (see Fig. 1), DC generation, antigen loading, *in vitro* maturation, and inoculation with or without adjuvant is an opportunity to enhance efficacy. DC vaccine research has therefore focused on expanding the available sources of DC and improving DC immunogenicity, optimizing the source and presentation of antigen, developing new immune adjuvants, and investigation of concomitant immunomodulation or chemotherapy. In this review we will discuss developments made in the last 5 y in each of these categories.

Source of dendritic cells

In a majority of immunotherapy clinical trials, DC are generated from peripheral blood mononuclear cells (PBMC) collected by

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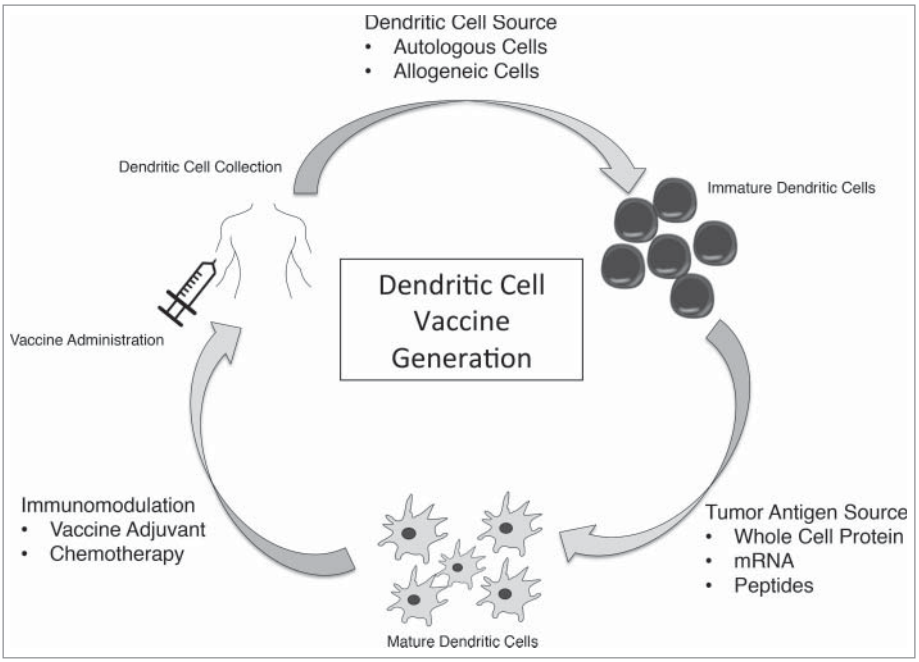

Figure 1. Dendritic Cell Vaccine Generation. DCV generation and administration is a multi-step process. A cell source for DC must be selected and DC generated, target antigen must be selected and dendritic cells exposed to the antigen for maturation, and finally DCV must be administered which can be done with concurrent immune modulators or vaccine adjuvants.

leukapheresis or phlebotomy. This usually results in consistent vaccine generation, but for patients who have recently received chemotherapy or those with CNS tumors who may require steroid therapy, generation of DC from a PBMC collection may not be feasible.[3,15] Because of the difficulty in generating DC from some patients, alternative sources of DC have been explored. Three studies have recently reported the generation of DC from novel cell sources in the pediatric population. A single patient with residual active leukemia following haematopoietic stem cell transplant (HSCT) was reported to receive an allogeneic DC vaccine derived from PBMC collected from her stem cell donor.[16] Our group reported a single patient with neuroblastoma whose DC were generated from a cryopreserved, G-CSF mobilized peripheral blood stem cell (PBSC) product,[3] and Nair reported the feasibility of generating DC from cryopreserved autologous PBSC products in patients with medulloblastoma.[17] This group was able to generate phenotypic DC from 3/5 samples and functional DC from 2/5 samples.[17] While this study met metrics for feasibility of DC generation, results are in line with previous data that the yield of functional DC may be lower from children with active tumors than from healthy adult donors. This is likely multifactorial and due to immunosuppression or tolerization by the tumor mass as well as previous myelo- and/or immunosuppressive therapy.[18,19]

PBSC could be an attractive source of DC because they can be collected prior to the onset of chemotherapy or even induced from an allogeneic source, bypassing the need to culture these cells from an immunocompromised host.[20] However, PBSC are also a problematic source of DC because GCSF mobilization can potentially skew DC to a DC-2/tolerogenic phenotype[21,22] making them a poor choice for an immunotherapy product. Several groups have generated DC from pluripotent stem cell lines,[23] induced pluripotent stem cells,[20,24] or embryonic stem cells.[25,26] In all cases, these DC were able to induce antigen specific cytotoxic T lymphocyte (CTL) or natural killer (NK) cell responses *in vitro*, and in 2 studies murine tumor models were used to show *in vivo* efficacy, defined by shrinkage of tumors and prolonged survival.[24,25] No human studies have used DC derived from pluripotent or embryonic stem cells. Finally, de Haar and group have reported a protocol for generating DC from a portion of a cord blood unit used for HSCT, such that patients could be vaccinated with allogeneic DC from their cord blood HSCT donor.[27] These alternative cell sources warrant further investigation, particularly regarding *in vivo* survival and immune response stimulation, because they could expand therapeutic options for these patients.

Antigen selection and loading

The selection of tumor antigen for loading onto DC is critical to maximize the likelihood of achieving an immune response. It is attractive to use a tumor specific antigen if one is known, but for some tumors no consistent tumor associated antigen (TAA) has been identified. Choices for antigen include HLA restricted epitopes, whole tumor lysate, or mixes of peptides from whole antigen. Each of these antigen sources have associated advantages and pitfalls and much research is done regarding the optimal antigen to stimulate the immune system for a given tumor.

Individual tumor antigen epitopes have the advantage of being well-characterized, immunogenic, and available from non-autologous sources. Nevertheless the use of individual epitopes limits immunotherapy to individuals with a specific HLA background. This problem can be overcome by using multiple overlapping peptides from a single protein, thereby providing an epitope library in a non-HLA restricted manner. Peptide mixes for some TAA are commercially available, although differences in composition of various lots could

impact vaccine immunogenicity. Examples of recently studied tumor specific antigens include the use of an HLA restricted Wilms' tumor 1 epitope in recurrent high grade glioma patients [28] and a non-HLA restricted pancreatic bile salt protein in pancreatic adenocarcinoma.[29] A group in Japan pulsed autologous DC with a mix of WT-I and WT-II to expand the applicability of this approach and demonstrated increased EFS and OS in patients who had positive DTH skin testing to either antigen after administration.[30] Our group has published a Phase I trial of DC vaccine targeting the cancer germline antigens (CGA) MAGE-A1, MAGE-A3, and NY-ESO-1 in children with solid tumors which were previously shown to upregulate these antigens in response to the demethylating chemotherapy agent decitabine (DAC). Patients were treated with DAC to upregulate CGA followed by injection of an autologous DC-vaccine pulsed with commercially obtained overlapping peptides derived from each of these 3 antigens,[3] permitting the enrollment of patients irrespective of their HLA background. We were able to demonstrate some immune responses and one heavily pretreated patient had a durable complete response.

If an immunogenic TAA has not been identified, one option is treatment with DC that have been pulsed with either whole cell protein or whole cell mRNA. Autologous tumor protein lysates have been used widely in pediatric DC immunotherapy.[5,7-11,13,14] Strengths of this approach include that fact that lysate is a reliable source of tumor antigen, particularly in tumors for which antigens have been poorly characterized. However, tumor tissue must be obtained, which limits this approach to those with measurable and resectable disease. This method also necessitates individualized vaccine production which takes time and may limit accessibility of therapy. Obtaining viable cells from autologous tumors may allow for some manipulation of these cells before lysate is generated. One possible manipulation is short term culture to isolate a cancer stem cell (CSC) population for selective CSC lysate pulsing. Because CSC are recognized to be capable of escaping traditional chemotherapy, the use of DC-based immunotherapy targeting antigens expressed by CSC could lead to better long term tumor control. However, there are concerns regarding theoretical generation of autoreactive T cells directed against stem cells in the tissue of tumor origin or in other stem cell populations. CSC antigen pulsed DC have been generated in a mouse model of breast cancer and re-inoculated with good efficacy and no evidence of toxicity to other stem cell populations.[31] Another group planned to study feasibility of producing autologous tumor cell lysates from patients with hepatocellular carcinoma. Vaccine production relied on short-term tumor cell culture. Unexpectedly, the success rate of tumor cell culture in their model was 100%, which they speculate may have been due to growth media selecting and propagating cells with stem cell properties. This feasibility trial showed no hepatotoxicity of DC vaccine with these hepatic "stem-like" cell lysates used to load DC.[32]

Another potential source of antigen is autologous tumor whole-cell mRNA. Electroporated mRNA drives expression of TAA in DC resulting in antigen presentation through MHC Class I molecules and could potentially result in more persistent presentation of tumor antigens than with protein pulsing. This antigen source, similar to whole cell lysate, offers the advantage of presenting a full range of tumor-relevant antigens. A Phase I trial in glioblastoma patients utilized mRNA isolated from autologous sphere-forming CSCs expanded in short term culture and demonstrated tumor specific T-cell proliferation after vaccination as well as increased progression free survival compared to historical controls.[33] Importantly, there was not any observed auto-immunity, particularly in eye (neural) tissue or myeloid stem cells.[33] In 2015, long-term follow-up of 30 melanoma patients who were treated with autologous mRNA-DC vaccine was reported. These patients had micrometastases but no measurable disease at the time of vaccination. At 6 y from the end of therapy, median survival had not yet been reached, and observed 2 y and 4 y survival rates of 93% and 70%, were at least 10% higher than historical controls at 4 y.[34] Interestingly, the EFS was not improved, but relapses were all early and these patients were effectively salvaged, leading to the excellent OS. Similar findings regarding lag time to vaccine response have been reported[4] and may indicate that time is needed for DC driven anti-tumor immunity to develop.

Since autologous tumor tissue may not be available in all cases, another source of tumor antigen being explored is allogeneic tumor cell lines. Although there are differences within cell lines for the same tumor type, the similarities to a patient tumor are likely to be greater than the differences, so it may be possible that DC loaded with lysates from a cell line would yield results similar to loading with autologous tumor, therefore bypassing the need for surgery. This approach was safe in a study of 8 vaccinated patients with recurrent brain tumors including 3 pediatric patients.[35] In addition, a post-hoc analysis in these patients indicated that patients with stable disease post-vaccine had an increase in IL-17α production, natural killer cells, and $CD8^+$ memory T-cells and a decrease in myeloid derived suppressor cells (MDSC), providing evidence for immune responses to common antigens as a possible explanation for disease stabilization in these patients. Though it did not reach significance, there were also lower CTLA-4 levels in patients with stable disease suggesting that this may be a potential target of immune modulation in future studies.

Some investigators have reported using multiple novel modalities in which an alternative source of DC and ubiquitous tumor antigens were used in combination. DC have been generated from pluripotent stem cells and since they can be passaged in culture, these DC can be directly transduced with tumor antigen DNA for stable expression, which can be presented on MHC class I and II molecules. This combination strategy utilizing a novel source of DC and stable transduction of common tumor antigen DNA was used in 3 recent studies[23-25] with demonstration of efficient and prolonged CTL stimulation. This strategy is a step toward generation of a generic, non-autologous vaccine, with DC possibly induced from human stem cells, transduced with common tumor antigen, and cultured and packaged for immediate availability. If feasible, this could ultimately expand the population for whom vaccine is available as well as shorten the time to availability. More study is required before it is ready for human trials.

Adjuvant for DC vaccines

For DCV efficacy, DC must migrate to a lymph node and activate effector cells, either $CD8^+$ T-cells or B-cells. In the right

milieu, DC can also activate $CD4^+$ helper T-cells and induce tolerance. Therefore, one very active area of research in DC immunotherapy is the development of adjuvants to stimulate DC function and/or specific effector populations *in vivo*. DC rely in part on signaling through toll like receptors (TLR) for maturation, resulting in expression of MHC Class I and II molecules and the secretion of pro-inflammatory cytokines.[36-38] Stimulation of TLR on DC can also facilitate the induction of Th1 immune responses.[39,40] In a review of several vaccine studies for malignant melanoma, Engel-Noerregaard reported significantly higher response rates in patients who received adjuvants as a part of their vaccine regimen.[41] Poly-ICLC (Hiltonol) is an inosine and cytidine-rich double stranded RNA that stimulates TLR3 and activates DC through a TLR-domain containing adapter inducing interferon-β (TRIF). It also stimulates CD8+ T cells and NK cells and increases interferon production. While it has primarily been used in combination with peptide vaccines, and not with autologous dendritic cells, it has been well-tolerated as a vaccine adjuvant in both children and adults[42-45] and due to its stimulatory effects on DC as well as effector cells, this agent is currently being used by our group in our ongoing DC vaccine trials in children with malignant solid tumors.

Other recently published work has focused on the use of novel adjuvants from natural sources,[46-49] known recall antigens,[50] tumor derived immunogenic proteins,[51] or proprietary costimulatory mixtures.[52,53] In one study of naturally occurring plant polysaccharides used in ancient Chinese medicine, intraperitoneal injections of extracts from *Antrodia cinnamomea* were shown to enhance DC activation *in vivo*, with increased T_H1 T cells as well as increased native $CD11c^+$ DC's in tumor draining lymph nodes.[47] In another study, vaccination with DC that were stimulated *in vitro* with a combination of extracts from *Astragalus membranaceous* and *Colonopsis pilosulae* resulted in increased tumor control in an *in vivo* murine breast cancer model.[46] Three additional studies showed that *in vitro* pre-treatment of tumor antigen pulsed DC with uric acid,[48] γ-glutamic acid,[49] or pancreatic adenocarcinoma upregulated factor (PAUF), a protein naturally secreted by human pancreatic carcinomas,[51] resulted in tumor shrinkage in murine tumor models. Co-treatment which removed or inactivated CTL abrogated tumor responses to these adjuvants, demonstrating that the mechanism of tumor death was dependent on pulsed DC activation of CTL. In three of these cases, adjuvant activity was definitively shown to activate the TLR pathway in DC.[47,49,51] More study is needed, however, because the role of TLR in DC activation has been extensively and recently reviewed[40,54] and one theme that has emerged is that TLR activation in murine models cannot be easily extrapolated to human models because murine and human DC constitutively express a different overlapping set of TLR.

DC, exposed to antigen can be matured *in vitro* or *in vivo*. Recent studies regarding *in vitro* maturation and DC activation include publications by a group in Brussels regarding clinical effectiveness in melanoma of DC that are electroporated with TriMix, a proprietary mixture of mRNA for CD40L, CD70, and a constitutively active TLR4, prior to antigen loading.[52,53]

A final area of study for vaccine adjuvants is co-injection of immunostimulatory compounds with the DC vaccine. Mitchell and group reported results of a randomized trial for adults with newly diagnosed glioblastoma who were treated with DC vaccine loaded with *Cytomegalovirus* phosphoprotein 65 (pp65) with or without pre-treatment with tetanus toxoid (Td).[50] They found that pretreated patients had increased migration of DC to vaccine draining lymph nodes as well as increased interferon- γ production in post-vaccine ELISPOT assays. In addition, they showed significantly improved progression free survival, with 3 patients alive and disease free at 3 y post-vaccine. Utilizing a mouse model that compared wild type to CCL3 knock-out mice, they showed increased DC migration to be dependent on CCL3. In addition, they showed the CCL3 to be produced by $CD4^+$ T-cells specifically activated by the Td recall response. This approach has not been tested in children but could be relevant to the pediatric population because tetanus vaccination is initiated at 2 months of age.

Because patients with cancer are often immunosuppressed from the tumor or their therapy these developments in adjuvant therapy to maximize DC function and effector cell activation are vital.

Concomitant immunomodulation

A consistent feature of DC immunotherapy studies is that *in vitro* activity of antigen-loaded DC in stimulating antigen-specific CTL responses does not clearly translate to an *in vivo* anti-tumor response. One possible explanation is that tumors are increasingly understood to generate an immunosuppressive milieu that induces T-cell anergy and an immunotolerant phenotype. Immunomodulators may have a role in changing this milieu to allow tumor infiltration by CTL and DC activation within the tumor itself. One attractive target for this approach is the PD-1/PD-ligand system. PD-1, when stimulated on the surface of T-cells induces antigen specific anergy or apoptosis.[55,56] Many tumor types as well as mature DC have been shown to express PD-ligand (PD-L1) on the cell surface.[55] DC found in tumor draining lymph nodes in an ovarian carcinoma model express high levels of PD-L1, and blockade of PD-L1 enhanced activation of CTL by DC and shifted the cytokines produced from a predominantly IL-10 producing T_H2 response to an IL-12 T_H1 response.[57] A study by Zhang et al looked at the effect of treatment with antibody against PD-L1 at various points in the DC vaccine process. This group demonstrated that treatment with α-PD-L1 antibody during DC culture increased proliferation and IL-12 expression of DC and that T-cells stimulated in the presence of α-PD-L1 secreted increased levels of interferon-γ.[58] In addition, they demonstrated that DC-vaccine against a PD-L1 expressing breast cancer model showed a statistically significant increase in tumor shrinkage when mice were co-treated with α-PD-L1.[58] While this group did not find any evidence of auto-immunity in treated mice, systemic inhibition of the PD-1/PD-L1 system is a concern because of the potential to block important mechanisms for self-tolerance in the normal immune system. This concern has led some groups to investigate ways of achieving a more focused silencing of PD-1/PD-L1.[59,60] In one study, DC from healthy volunteers were infected with a lentiviral vector encoding short hairpin RNA (shRNA) for PD-L1 which resulted in abrogation of PD-L1 production. They found no change in the

standard DC phenotype except loss of expression of surface PD-L1, but cells treated in this way had increased ability to stimulate T-cell proliferation, secretion of IL-12 and interferon-γ, and *in vitro* tumor cell killing.[60] A study by van der Waart, et al. investigated this further using an *in vivo* murine AML model.[59] In this study DC derived from PBMC were treated with short interfering RNA (siRNA) against PD-L1 and or PD-L2 and were then shown to increase *in vitro* proliferation of antigen specific $CD8^+$ T-cells by as much as 20-fold. Further, infusion of these Ag-specific CTL simultaneously with vaccination of PD-L1 silenced DC induced an increased and sustained antigen-specific CTL response.[59] These modifications to PD-1/PD-L1 expression on DC did not result in any systemic toxicity.

Another mechanism to circumvent the immunosuppressive effects of the tumor microenvironment is IL-10 suppression. IL-10 is known to decrease MHC-I expression, inhibit NK cell activity, and decrease important DC costimulatory molecules. An α-IL-10 antibody administered 24 hours before DC vaccine was able to increase NK responses and was associated with a statistically and clinically significant decrease in tumor growth and increase in survival in a murine breast cancer model.[61] This protocol also made use of a single low dose of cyclophosphamide to decrease regulatory T-cells, but this intervention alone did not result in a change in tumor growth in the absence of the α-IL-10 antibody.

Dasatinib is a multi-tyrosine kinase inhibitor with known efficacy in BCR-abl fusion driven haematopoietic malignancies but with only modest effects in other tumors. This agent inhibits cKIT and SRC kinases known to play a role in maintenance of MDSC and T_{regs} respectively. Low dose oral dasatinib in combination with anti-melanoma DC-vaccine significantly increased tumor infiltrating CTL and $CD11c^+$ DC, decreased signaling through hypoxia mediated pathways, increased intratumoral expression of pro-inflammatory cytokines and chemokines, and resulted in a striking decrease in tumor growth.[69] Taken as a group these studies show that concomitant modulation of immunosuppressive pathways may enhance DC-mediated anti-tumor immune response.

Tumors are known to induce immunosuppression through other pathways including modifications of L-arginine metabolism[62] and induction of indoleamine 2,3 dioxygenase (IDO).[63-65] While no recent work has combined modulation of these systems with dendritic cell vaccines in a preclinical or clinical model, one group demonstrated that IL-6 drives the arginase pathway to induce DC dependent $CD4^+$ T cell dysfunction,[66] implying that a drug that interferes with IL-6 such as tocilizumab (α-IL-6R) or situxilizumab (α-IL-6) may boost DCV efficacy. In addition, an *in vitro* model of monocyte derived DC induction of anti-leukemic T-cell activity demonstrated that PGE_2 used in DC maturation increased production of IDO and that co-culture with an inhibitor of IDO, Levo-1-methyl-tryptophan (L-1-MT) significantly increased DC driven T-cell proliferation.[67] L-1-MT has been used to inhibit IDO in a mouse glioma model,[68] but has not been used in humans and is not commercially available as a drug.

Future directions

Many of the studies regarding DC vaccine biology in the last 5 y have been primarily directed at adult malignancies, but most of these strategies could be easily translated into pediatric trials (see Table 1). Because children are small, they are often

Table 1. Leading Clinical and Preclinical candidates with potential to increase DCV efficacy. Definitions: *DC gen* - DC generation; *DCV prod* - DCV production; *caTLR4* - constitutively active TLR4; *shRNA* - short hairpin RNA; *siRNA* - short interfering RNA; *TKI* - tyrosine kinase inhibitor.

Authors[Ref]	Preclinical/Clinical	DCV Stage	Target	Key Results	Applicable to Pediatrics
Nair, et al.[17]	Preclinical	DC gen	New DC source	Feasible to create DC from cryopreserved autologous stem cell apheresis product	Yes
Benteyn, et al.[53]	Preclinical	DCV prod	DC activation	*In vivo* DC maturation with proprietary co-stimulatory molecules – TriMix (CD40L, CD70, caTLR4)	Unknown
Olin, et al.[35]	Clinical	DCV prod	Novel Antigen	DC loaded with allogeneic tumor cell line lysates were tolerated, best response SD in 1 patient	Likely
Van Nuffel, et al.[52]	Clinical	DCV prod	DC activation	Durable T-cell and clinical response in chemorefractory melanoma patient treated with TriMix	Unknown
Mitchell, et al.[50]	Clinical	Adjuvant	Effector T-cells	↑ DC migration to draining lymph nodes, ↑ interferon- γ production post-vaccine, improved PFS	Yes
Rossowska, et al.[61]	Preclinical	Immuno-modulation	IL-10	Inhibition of IL-10 with DCV ↑ tumor growth inhibition in a murine model	Unknown
Ge, et al.[58]	Preclinical	Immuno-modulation	PD-1/PD-L1	PD-L1 blockade during DC generation/injection ↓ tumor growth and ↑ PFS in murine breast cancer model	Likely
Wang, et al.[60]	Preclinical	Immuno-modulation	PD-1/PD-L1	Transduction of shRNA against PD-L1 into DC ↓ PD-L1 expression, ↓ IL-10 production, ↑ IL-12	Unknown
Van der Waart, et al.[59]	Preclinical	Immuno-modulation	PD-1/PD-L1	siRNA silencing PD-L1 and L2 in DC ↑ *in vitro* T-cell priming and *in vivo* CD8+ T-cell responses	Unknown
Lowe, et al.[69]	Preclinical	Immuno-modulation	Effector T-cells	TKI dasatinib combined with DCV ↓ tumor growth and ↑ OS compared to either modality alone	Likely

transplanted with umbilical cord blood, and studies using cord blood derived DC's in transplant patients or in patients who may have their own cord blood banked could be undertaken. Regarding concomitant immunomodulation strategies, while α-PD-L1 medications have pediatric dose finding studies ongoing and are not yet ready to be routinely administered to children, dasatinib has been through Phase I testing in children[70] and is currently used in pediatric patients for leukemias with associated BCR-abl translocations.[71,72] There are indications that CTLA-4 blockade may be beneficial in high grade gliomas, and ipilumimab, the α-CTLA-4 medication has been used in pediatric Phase I trials.[73] Tocilizumab has a defined safety profile in children with rheumatologic disease[74,75] and could be moved into cancer clinical trials. All of these drugs could be readily tested in combination with DC vaccines if pre-clinical data show benefit. Because there seems to be a lag between administration of a DC vaccine product and tumor response, and because DCV are so well tolerated, it is reasonable to consider moving the timing of DC vaccine therapy to a relapse prevention strategy in which DC are harvested before chemotherapy and administered at the conclusion of cytotoxic therapy when the tumor is clinically in remission.

In summary, recent advances have been made with novel adjuvants and concomitant immunomodulation increasing immunogenicity and effector cell stimulation by dendritic cell vaccines, and advances in antigen and DC source selection will make this therapy available to more patients. Currently, there are more than 30 open clinical trials using DC cell based immunotherapy in pediatric and adult cancers. Lessons learned from these trials and ongoing clinical and preclinical testing should inform the future evolution of immunotherapy trials.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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