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New approaches to the development of adenoviral dendritic cell vaccines in melanoma

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Abstract

Considerable research in the field of immunotherapy for melanoma has demonstrated that this tumor type can be responsive to therapeutic immune activation strategies. In early clinical trials, vaccine strategies using dendritic cells (DCs) and adenovirus (Ad) vectors (AdVs) were safe and immunogenic, and induced clinical responses in a minority of patients. Research from the past several years has yielded an improved mechanistic understanding of DC biology, AdV effects on DCs and the crosstalk that occurs between antigen-loaded DCs and specific lymphocyte subsets. This knowledge base is being combined with technological advances in cytokine delivery, AdV design and in vivo DC targeting. These developments are leading to novel AdV-transduced DC-based therapeutic modalities that may further advance melanoma immunotherapy. Interactions between AdVs and DCs, initial clinical trial results, and new developments in DC engineering and in AdV biology are reviewed.

Keywords

Adenovirus; cancer vaccine; costimulation; cytokine; dendritic cell; melanoma

Introduction

It has been estimated that there will be 68,130 new cases of invasive melanoma and 8700 deaths from this disease in the US during 2010 [1]. The incidence of melanoma has increased significantly during the past several decades from an annual incidence of 6% in the 1970s, and is now increasing at a rate of 3% annually. While the 5-year overall survival for this disease is 91%, after melanoma has spread regionally, the 5-year survival decreases to 62%, and with spreading to a distant site, the 5-year survival rate is only 15% [1]. The median age at presentation of melanoma is 47 to 48 years and, until recently, no therapy had demonstrated an increase in overall survival in patients with unresectable metastatic disease. Support for immune-based approaches that have potential to impact on disease significantly was provided in a recent clinical trial by Hodi *et al* [2], in which ipilimumab (Bristol-Myers Squibb/Medarex) was demonstrated to improve survival in patients with metastatic melanoma.

Immunotherapy approaches have mainly been used in the treatment of melanoma because this disease is often viewed as an ‘immunogenic’ tumor type. Several immunotherapy approaches have progressed into clinical testing, including cancer vaccines, the adoptive

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transfer of tumor-specific T-cells, antibodies and cytokines. This review focuses on cancer vaccines for melanoma in which dendritic cells (DCs) and adenovirus (Ad) vectors (AdV) are used to promote antitumor immunity. Significant progress has been made in developing engineered DCs carrying AdV-encoded tumor antigens, cytokines and costimulatory molecules. Moreover, novel vector backbones and new DC-targeting approaches are progressing toward clinical testing.

Dendritic cells

DCs are the key physiological stimulators of naïve and memory lymphocytes [3–5]. The presentation of antigen epitopes by a sufficiently matured DC occurs in optimal immunostimulatory conditions, with high levels of MHC class I and II, costimulatory (CD80, CD86 and CD40) and adhesion (CD54) molecule expression, and optimal amounts of cytokines (IL-12p70) and chemokines (CCL22/MDC, CXCL8/IL-8, CCL19/MIP-3β) produced [6]; these signals stimulate the immune system to induce a protective type 1 cytotoxic response against the antigen. This process is in contrast to antigen presentation that occurs in the tumor microenvironment, which lacks adequate costimulation or the production of appropriate cytokines, resulting in the downregulation of immune responses [7]. In animal models, DCs pulsed with tumor antigen-derived peptide epitopes have been demonstrated to induce antitumor responses [8–10]. Moreover, this DC-based cancer vaccine strategy has generated clinically relevant responses (including complete clinical responses) in patients with low grade lymphoma, multiple myeloma, advanced malignant melanoma or prostate cancer [11–17].

Immunotherapy clinical trials in patients with metastatic cancer [18], and with metastatic melanoma in particular [19], have demonstrated that DC-based trials result overall in a 7% objective response rate and a 9.5% tumor regression rate, respectively. These early trials provide strong support to DC immunogenicity and their potential for clinical use. However, research with DCs has identified a high degree of plasticity, depending on culture conditions and maturation treatments [20]. Defined culture additives and maturation cocktails of cytokines and TLR agonists can potently modulate the biology of the resultant DCs; standardized cells produced under defined culture conditions can better instruct the lymphocytes that they interact with to become cytotoxic, T-helper type 1 (Th1), Th2 or regulatory in function [20].

Adenovirus vectors

AdVs are an established gene delivery vehicle with an extensive safety record. Ads are well characterized [21], and several classes of replication-defective vectors have been developed and tested. Although the presence of pre-existing neutralizing antibodies to this vector (from environmental exposure) may be a concern, in mice pre-immunized with AdV (to induce circulating neutralizing antibodies) and subsequently immunized with AdV-transduced DCs (AdV/DCs), the same high level of antitumor immunity was induced, regardless of neutralizing antibody levels [22,23]. Conversely, systemic delivery (ie, intravenous, intramuscular or subcutaneous) of virus was strongly inhibited by pre-existing anti-AdV immunity, and the resultant level of *in vivo* infection of host DCs was reduced, as were T-cell responses [24].

Adenovirus vector infection and signaling

The entry of AdVs into DCs requires two sets of interactions between the virus and the host cell. First, AdVs from the Ad2 and Ad5 serotypes attach to cells by the binding of the knob domain of the fiber protein to the coxsackie-Ad receptor (CAR) present on the host cell [25,26]. This binding is followed by a secondary interaction in which the penton base

protein engages the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, stimulating cell signaling and resulting in entry of the AdV into the DCs [26,27]. Following internalization of the AdV, the virus localizes initially in early endosomes and then in late endosomes; the virus later escapes to the cytoplasm, where transgene expression occurs [28]. The linear viral dsDNA genome is then transported to the nucleus, but is maintained extrachromosomally. Although DCs express low levels of CAR, the cells do express high levels of integrins, as well as the heparin-sensitive receptor that is recognized by a distinct segment (ie, the shaft) of the AdV fiber, allowing DCs to be transduced efficiently with AdVs [29,30]. High AdV multiplicities of infection yield > 95% transduction efficiency, and result in high levels of transgene expression and no cytopathic effects [31]. TLRs do not appear to be involved in AdV-mediated signaling in human DCs; signaling is instead induced by a nucleic acid-sensing mechanism that recognizes the viral dsDNA in the cytoplasm and induces IFN regulatory factor 3 (IRF3) activation [32]. In addition, myeloid DCs also activate PKR and PI3K signaling pathways in response to AdV infection, but the activation mechanism remains unclear [33,34].

Adenovirus vector-induced changes in dendritic cell phenotype and function

AdV transduction has a profound impact on human DC biology and function. Immature monocyte-derived DCs transduced with high levels of AdV (multiplicity of infection of ≥ 500 PFU/cell) become more mature, as demonstrated by: (i) increased levels of CCR7, CD83, CD86 and HLA-DR expression; (ii) increased production of IL-8, TNF, IL-15, IL-12p70, IFNα and IFNβ; (iii) decreased secretion of IL-10 and IL-13; and (iv) reduced antigen uptake via macropinocytosis [28,35–40]. AdV-infected DCs also secrete elevated levels of chemokines, such as CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL19/MIP-3β, CXCL8/IL-8, CXCL9/MIG and CXCL10/IP-10, allowing the cells to recruit several different lymphocyte subsets [Vujanovic L, Butterfield LH: unpublished data]. In addition, AdV infection induces specific changes to the antigen presenting machinery in human DCs, including increased expression levels of the peptide transporters TAP-1 and TAP-2, and the HLA class I peptide-loading chaperone ERp57 [39]. These data identify functional and molecular changes in AdV/DCs that explain why these cells are efficient at stimulating antigen-specific, IFNγ -producing T-cells [28,35–40]. E1-deleted, replication-deficient AdV/DCs also stimulate T-cells that recognize AdV epitopes (which are recall antigens for most individuals) [41–43], perhaps because of the leakiness of AdV gene expression or because of the processing of preformed viral proteins transferred at transduction [42].

Antigen-engineered dendritic cells

The successful engineering of DCs with recombinant AdV, and the clear superiority of this approach to traditional physical methods of transfection, such as with calcium phosphate and lipids, was demonstrated in 1997 [31]. Since this time, AdVs have been used as vectors to deliver antigens, cytokines, costimulatory molecules and other transgenes to DCs. Other methods have been used for the transfer of genes to DCs [44], including, most recently, lentivirus vectors [45,46] and optimized physical methods such as electroporation [47]. Tumor antigen-engineered AdV/DCs are capable of processing and presenting antigen epitopes in the context of both MHC class I and II molecules. The continuous display of multiple peptide epitopes is restricted by the patient's own MHC alleles, and has the advantage of generating polyclonal T-cell responses. Broad and potent activation of multiple CD8+ and CD4+ T-cell specificities [48,49] can be induced by AdV/DCs, and the responses obtained are superior to protein-loaded DCs [50]. While murine models have suggested CD8+ T-cells are the most important antitumor effectors, considerable data also support the

significance of CD4+ helper T-cells and the activation of innate immunity to cooperatively induce an effective antitumor response [51]. The provision of antigen-specific mediated by CD4+ helper T-cells may improve the function of CD8+ effector CTLs. For example, antigen-specific activation of CD4+ helper T-cells resulted in the potent activation of CD8+ T-cells, which are then capable of expansion, differentiation and secondary rounds of proliferation [52].

DCs transduced with specific antigen genes process and present antigenic peptides for at least 10 days, while DCs pulsed with the immunodominant peptides present at 50% of the maximal level by day 2 post-pulsing; the maximal CD8+ MART-1-specific T-cell response is superior for the AdV/DCs [53]. Many comparisons of exogenous peptide pulsing versus antigen transfection in DCs have been conducted, and support the superiority of DC transfection with full-length antigen genes for optimal peptide-specific T-cell activation [54–57]. Plasmid DNA transfection can be effective when using efficient delivery methods [58], including nucleofection, but in a direct comparison of nucleofection versus transduction with a fiber-modified Ad5/35 AdV, the fiber-modified Ad5/35 AdV was superior [59]. An additional improvement in antigen transduction may be the engineering of DCs to express multiple antigens, resulting in the stimulation of multi-antigen CD8+ and CD4+ T-cell responses [Butterfield LH, Vujanovic L, Kirkwood JM: unpublished data]. This strategy would theoretically allow for the more effective treatment of tumor lesions by also promoting determinant spreading, which has been positively correlated with the survival of patients with cancer [15,16,60]. The stimulation of T-cell responses with multiple antigens may be critical to combat the formation of tumor variants that exhibit antigen- and antigen processing machinery-loss [61–67].

Clinical trials of adenovirus vector-transduced dendritic cells

AdV/DCs have progressed to clinical testing [68,69]. In an AdV/DC clinical trial in which DCs were transduced with both AdVMART-1 (MART-1 is a protein antigen that is present on melanocytes) and AdVgp100 (gp100 is a melanoma-associated antigen), 1 of 17 evaluable patients had a complete response [69] and 3 out of 12 patients with vitiligo were reported [68]. In a second trial testing AdVMART-1/DCs in patients (n = 23) with late-stage melanoma [70], the vaccine was safe and immunogenic, and led to activation and expansion of the desired MART-1-specific CD8+ and CD4+ T-cells. In addition, NK cells were activated in a subset of patients and expansion of regulatory T-cells was not detected. Clinical responses for the patients (n = 14) receiving all three vaccines were 1 patient with a suspected complete response, 4 with stable disease (27 to 42 months in duration), and 1 who became eligible for resection and subsequently remained without disease (> 58 months) [70]. These trials demonstrated that ‘first-generation’ AdV/DC vaccines were safe, immunogenic and exhibited clinical activity, and that the use of such vaccines was feasible.

Innate immune activation mediated by adenovirus vector-transduced dendritic cells

The ability of AdV/DCs to generate robust antigen-specific protective antitumor CD4+ and CD8+ T-cell responses has been well characterized; however, the role of NK cells in this response is less clear. The effectiveness of AdV/DC-based vaccines may, ultimately, depend on the ability of AdV/DCs to crosstalk with NK cells and to activate, polarize and bridge innate and adaptive immunity [71–74]. DCs and NK cells are essential components of the innate immune system that interact and reciprocally regulate each other, and induce an enhanced polarization of type 1 cytokine secretion [73–76]. Activated NK cells induce increases in the expression of maturation markers and the secretion of IL-12p70 in DCs [73–76]. Reciprocally, matured DCs induce the expression of activation markers, enhance IFNγ

secretion, perforin-mediated tumoricidal activity and proliferation in NK cells, and stimulate the ability of such cells to control *in vivo* viral infections and tumor growth [77]. This early cellular crosstalk is believed to be an essential regulatory immune mechanism that bridges innate and adaptive immune functions, and defines the quality and magnitude of antiviral and antitumor immune responses.

The ability of human AdV/DCs to activate NK cells was tested directly *in vitro*. AdV/DCs encoding *Escherichia coli* β-galactosidase (LacZ; a biologically inert protein) activated resting NK cells effectively, without enhancing AdV/DC susceptibility to NK cell-mediated killing, as measured by induced increases in CD69 expression (ie, a NK cell activation marker), IFNγ secretion, cell proliferation and killing of tumor cell targets *in vitro* and *in vivo* [40]. AdV/DC-mediated activation of NK cells required cell-to-cell contact, and was mediated by transmembrane TNF and *trans*-presented IL-15 present on the surface of DCs [40]. AdV/DCs also recruited both major subsets of NK cells effectively via CXCL8/IL-8 and CXCL10/IP-10 secretion that, coupled with their ability to activate NK cells, makes them potent immune activators of not only adaptive, but also of innate immune responses [Vujanovic L, Butterfield LH: unpublished data].

Cytokine-engineered dendritic cells

The maturation of DCs with cocktails of reagents results in cytokine production. However, in order for reproducible, constitutive and high levels of production of specific cytokines to be obtained from DCs, AdV transduction has been used. Several cytokines and chemokines known to be important mediators of antitumor immune responses have been tested in gene therapy protocols through the use of *ex vivo* gene-engineered DCs. Some of the cytokines and chemokines tested include IL-12, IL-15, IL-18, TNF and CCL21/6Ckine [73–82].

IL-12p70 and IL-18 as immunotherapeutic agents

IL-12 and IL-18 are two cytokines that are of particular interest to tumor immunologists because of their ability to promote synergistically IFNγ secretion from and proliferation of CD4+ effector T-cells and NK cells [78,79,83,84]. IL-12 is a heterodimeric, multifunctional cytokine with properties that bridge innate and adaptive immunity. This cytokine acts as a key regulator of cell-mediated immune responses, and induces type 1 immunity by stimulating NK cells and T-cells to produce IFNγ, to proliferate and to exhibit cytolytic activity [85]. In addition, IL-12 inhibits angiogenesis by promoting IFNγ-inducible genes and lymphocyte-endothelial cell crosstalk [86–88]. While type 1 immune responses have been associated with spontaneous or therapy-induced regression of tumor lesions [89,90], tumor-infiltrating lymphocytes isolated from patients with progressive lesions have been generally reported to exhibit dominant Th2-type (ie, secrete IL-4 and IL-5) or regulatory (Th3)-type (ie, secrete IL-10 or TGFβ1) responses [89–91]. Therefore, the type 1 immunity-skewing properties of IL-12 have provided the rationale for exploiting this cytokine as an anticancer agent.

IL-18 is a member of the IL-1 cytokine superfamily that has an important role in regulating immune responses. IL-18 is produced by APCs (DCs and monocytes), as well as by the pituitary gland, keratinocytes and osteoblasts, and adrenal cortical, intestinal epithelial and Kupffer cells (ie, phagocytes lining the hepatic sinusoids) [92]. While IL-18 was characterized initially as an IFNγ-inducing factor [93], later studies demonstrated that IL-18 is a unique cytokine that is capable of inducing either Th1 or Th2 polarization, depending on the type and context of stimuli, the cytokine priming milieu and underlying genetic influences [94]. Furthermore, IL-18 displays chemoattractant properties for both myeloid- and plasmacytoid-derived DCs [95,96]. IL-12p70 induces T-cell surface expression of the

IL-18 receptor (IL-18R) by naïve T-cells [97], while IL-18 potentiates the differentiation of Th1 cells initiated by IL-12 [98].

IL-12p70 has been tested using various administration methods for its ability to enhance Th1-type responses, and has achieved considerable immunostimulatory results *in vitro* and *in vivo* [85]. Furthermore, IL-12 combined with other immunotherapy approaches, particularly when administered with IL-18, has demonstrated improved immunostimulatory results compared with IL-12 alone. However, in clinical trials, systemically administered recombinant IL-12p70 (rIL-12p70) displayed unacceptable toxicities, including fever/chills, fatigue, nausea, vomiting, headache, anemia, neutropenia, lymphopenia, hyperglycemia, thrombocytopenia, hypoalbuminemia and even death [99–104]. Studies in mice demonstrated that these toxicities were mainly caused by IFNγ overproduction by NK cells [105]. Moreover, IL-12-mediated toxicity was particularly exacerbated with the coadministration of recombinant IL-18 (rIL-18) [106]. Studies in mice also demonstrated that simultaneous administration of rIL-12 and rIL-18 led to severe systemic inflammation, as a result of IFNγ secretion by NK cells, and 100% mortality, and these effects were STAT4-dependent [106].

IL-12p70 and IL-18 gene therapy delivered intratumorally through ex vivo gene-engineered dendritic cells

One possible approach to prevent systemic toxicity and to minimize toxic effects is to use gene transfer methods to limit IL-12p70 and IL-18 production within the tumor environment. This strategy has demonstrated increased efficacy and safety profiles in several studies and trials [107–109]. For example, in a phase I clinical trial in which recombinant Ad-transduced, IL-12p70 gene-modified autologous DCs were delivered intratumorally to colorectal, hepatic or pancreatic carcinomas, treatment was well tolerated with limited toxicities observed (lymphopenia and fever occurred in the majority of patients) [108]. However, objective clinical responses in the treated patients were limited. Mouse models have also demonstrated that the intratumoral delivery of AdV/DCs encoding IL-12p70 (under the control of a constitutive promoter), particularly when co-transduced with an AdV encoding IL-18, promoted enhanced Th1-type immunity and determinant spreading in the curative antitumor CTL repertoire, and acute tumor rejection [82,110,111]. While DC-based IL-12p70 gene therapy displayed potent efficacy, the sustained high levels of IL-12p70 and IL-18 could potentially cause unwanted toxicities, as well as cytokine-dependent limitations in DC migration after intratumoral administration [81,82,110,111].

The RheoSwitch therapeutic system: A novel method of controlling transgene expression in adenovirus vector-transduced dendritic cells

To circumvent concerns regarding sustained high levels of IL-12p70 with gene therapy approaches, the INXN-3001 plus INXN-1001 combination regimen (Intrexon) that incorporated a novel AdV encoding a conditional IL-12p70 expression system, which used the RheoSwitch therapeutic system (RTS) for the regulatory control of long-term therapeutic gene expression, was developed and tested in a subcutaneous B16 melanoma mouse model [112]. The RTS approach incorporated the rAd.RheoIL12 AdV in which the mouse IL-12p70 subunit genes were placed under the control of an inducible promoter that was activated by a diacylhydrazine small-molecule ligand [113]. Using this system, transgene expression was controlled effectively in transduced DCs (DC.RheoIL12) after intratumoral injection by regulating administration of the ligand. DC.RheoIL12 cells secreted significant amounts of IL-12p70 in a tightly regulated manner *in vitro*, and *in vivo* the treatment benefit of DC.RheoIL12 delivered into B16 lesions was strictly ligand-dependent [113]. The unique safety feature of DC.RheoIL12 was accentuated when this treatment was combined with IL-2: TNFα-associated toxicity was ameliorated by halting ligand administration [113]. The

use of the RTS approach can delay the activation of IL-12p70 production by injected DCs, potentially allowing the cells to acquire tumor antigens and to migrate to a tissue-draining lymph node, as well as providing a mechanism to inhibit IL-12p70 production if toxicity is observed.

Engineering dendritic cells to express high levels of costimulatory molecules

Costimulatory molecules expressed on the surface of DCs are considered to provide 'signal 2' (where 'signal 1' is the MHC/antigen complex), which is required for proper activation of adaptive immunity. The lack of 'signal 2' induces CD4+ and CD8+ T-cell anergy. Mature DCs express high levels of the costimulatory CD80, CD86 and CD40 molecules, which are critical for immunity in most models studied. The CD40 ligand (CD40L) is expressed on activated T-cells, and the CD40-CD40L signaling pathway promotes DC and T-cell crosstalk to facilitate full maturation of DCs. CD40L in several forms has been used to activate DCs *in vitro*, but an alternative strategy is to engineer DCs to express CD40L directly, which allows for strong signaling within DCs. This strategy has been used to activate DCs to secrete high levels of IL-12p70, migrate toward CCL19/MIP-3β and activate MART-1-specific T-cells [114]. Such DCs could gather antigen *in vivo* following intratumoral injection, or be co-transduced with an antigen-encoding AdV [114]. CD40L–engineered DCs were observed to be strong IL-12p70 producers, to express high levels of CD80, CD86, CD83 and IL-10, and to lead to reduced T-cell activation [115]. As noted, DCs are exquisitely sensitive to environmental signals, and seemingly minor methodological changes can impact the resultant DC-lymphocyte crosstalk [20].

Adenovirus vector-transduced dendritic cells engineered to secrete chemokines

CCL21/6Ckine is constitutively expressed in human lymph nodes, spleen and appendix and attracts CCR7-positive lymphocytes and DCs to lymph nodes [116]. *In vitro*, DCs transduced with AdV-encoded CCL21/6Ckine expressed high levels of this chemokine, resulting in the migration of lymphocytes to the DCs [117]. Such chemokine-engineered DCs have been tested with and without antigen loading in a B16 melanoma mouse model model [118], and were observed to induce the migration of CD8+ and CD4+ T-cells, as well as DCs. This strategy is now being tested clinically in patients with metastatic melanoma [119].

Novel adenoviral vector strategies for engineering dendritic cells

There are many innovative approaches in preclinical development that use combinations of AdVs and DCs in various disease models, including melanoma models. However, new strategies are required to improve viability, efficacy and targeting of AdV/DC vaccines.

Alternative adenovirus vector serotypes

Given the prevalence of pre-existing immunity to the commonly used Ad5 vectors, different Ad serotypes have been tested for use with DCs in order to determine their transduction efficiency, immunogenicity and downstream effects on DC and T-cells. In one *in vitro* study, chimeric Ad5/Ad35 vectors in which the fiber region of Ad5 was substituted with the Ad35 region were tested [120]. These vectors exhibited increased efficiency with respect to DC transduction and subsequent T-cell activation, while maintaining positive effects on DC maturation [120]. In a second study, Ad5/35 and Ad35 vectors were compared in human CD46 transgenic mouse-derived DCs. Ad35 serotype vectors promoted a stronger immune

response compared with Ad5 vectors [121], suggesting that antitumor immunity might be enhanced for Ad35-based approaches. A study of neutralizing anti-Ad antibodies in humans in Africa and in the US demonstrated the presence of high levels of circulating antibodies to Ad5, but lower levels to Ad35, indicating that, as expected, Ad5 exposure is more prevalent [122]. While this observation clearly impacts systemic AdV delivery, the impact of neutralizing antibodies on AdV/DCs may be minimal [22,23]. Ad3 vectors that use CD80 and CD86 costimulatory molecules as surface receptors have also been tested [123]; the use of these costimulatory molecules potentially makes these vectors naturally tropic for B-cells and DCs when administered *in vivo*.

Efficient targeting of adenovirus vectors to dendritic cells

Another approach to improve the transduction efficiency of DCs is to use CD40 for the specific targeting of AdVs to DCs. For example, the use of a bispecific antibody (specific for CD40 and the fiber-knob of AdV) improved transduction efficiency and human DC maturation *in vitro* [29]. This approach was tested in a subsequent study in human skin explants, and demonstrated increased efficiency and potential as an *in vivo* DC targeting strategy [124]. In addition, the targeting of AdVMART-1 to both myeloid and plasmacytoid DCs in human lymph node cell suspensions and subsequent activation of MART-1-specific T-cells was demonstrated [125]. Other targeting approaches that can improve *in vitro* transduction efficiency and that may be useful for *in vivo* targeting include the use of the FcγR1 (CD64) via a bispecific antibody approach [126], or modifying the CAR with a CAR-TAT (HIV TAT protein transduction domain) adapter molecule [127].

Using adenovirus vectors for improved dendritic cell-targeting to tumors and improved antigen release

Notably, AdV CCL19/MIP-3β can induce not only lymphocyte, but also DC migration toward the engineered cells [128]. An AdV encoding CCL5/RANTES has been tested in murine tumor models [128] and, in addition to increasing the levels of DC migration toward tumors, macrophages, NK cells and CD8+ T-cells, the engineered cells also migrated toward tumors. Furthermore, oncolytic AdVs, which replicate selectively in tumors and which can lead to tumor lysis, are also being used for the *in vivo* release of tumor antigens that can then be taken up by host DCs, leading to the activation of adaptive immunity [129]. The oncolytic AdV strategy has been combined with immune modulatory molecules (eg, IL-12p70 and 4-1BBL) to promote DC infiltration and antitumor immunity, as evaluated in a B16-F10 tumor model in mice [130].

Conclusion

Initial clinical trials of antigen-engineered AdV/DC vaccines in patients with melanoma have demonstrated that these agents are safe and immunogenic, and can promote potent and durable clinical responses in a minority of patients. Moreover, novel strategies involving multiple antigens, cytokines and chemokines, regulatable promoters and improved DC targeting are being translated to patients. These new approaches are based on improved knowledge of DC biology, AdV effects on DCs and lymphocyte activation.

There are major opportunities in the area of DC-based vaccines. First, there is a need to implement immunological monitoring in clinical trials in order to understand the effects of AdVs, full-length antigens, and cytokines or chemokines encoded by the vector on the diverse lymphocyte populations being activated. Second, it is important for specific DC culture and maturation conditions to be combined rationally with AdVs in order to optimize the antigen presentation and lymphocyte activation abilities of these cells. Third, the most cost-effective and broadly applicable approach would be to use a virus that is capable of

specific *in vivo* targeting of DCs; therefore, current approaches are in need of further development. Ultimately, these studies are expected to provide immunotherapy approaches for the treatment of melanoma that exhibit greater efficacy. These strategies may lead to stand-alone therapies that are useful in early-stage disease, or may be used as part of a combination strategy that includes the adoptive transfer of lymphocytes, antibodies or other types of interventions in more advanced disease.

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