Potent Antitumor Immunity Generated by a CD40-Targeted Adenoviral Vaccine

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Abstract

In situ delivery of tumor-associated antigen (TAA) genes into dendritic cells (DC) has great potential as a generally applicable tumor vaccination approach. Although adenoviruses (Ad) are an attractive vaccine vehicle in this regard, Ad-mediated transduction of DCs is hampered by the lack of expression of the Ad receptor CAR on the DC surface. DC activation also requires interaction of CD40 with its ligand CD40L to generate protective T-cell–mediated tumor immunity. Therefore, to create a strategy to target Ads to DCs in vivo, we constructed a bispecific adaptor molecule with the CAR ectodomain linked to the CD40L extracellular domain via a trimerization motif (CFm40L). By targeting Ad to CD40 with the use of CFm40L, we enhanced both transduction and maturation of cultured bone marrow–derived DCs. Moreover, we improved transduction efficiency of DCs in lymph node and splenic cell suspensions in vitro and in skin and vaccination site–draining lymph nodes in vivo. Furthermore, CD40 targeting improved the induction of specific CD8+ T-cell responses which in some cases were associated with long-term survival (4).

Introduction

Melanoma vaccines based on ex vivo prepared autologous dendritic cells (DC) have been reported to induce specific T-cell responses which in some cases were associated with improved survival (1–3). In spite of its first clinical successes, serious drawbacks are inherent to this approach. The ex vivo culture and antigen loading of DCs according to cGMP guidelines and on a per-patient basis are laborious, time consuming, and introduce interdonor variability that may complicate the interpretation of clinical outcome. Furthermore, ex vivo modified DC migrate poorly in vivo, affecting the efficacy of the vaccine (4). In vivo targeting of tumor-associated antigens (TAA) and/or activating agents to DC presents an attractive alternative (5). The approaches used for in vivo targeting of antigens to DCs range from viral or liposomal targeting to the conjugation of antigens directly to DC-binding ligands or monoclonal antibodies (mAb; refs. 5–12). Targeting of DC receptors such as DC-SIGN (13), DEC-205 (8), and CD11c (9) required the incorporation of additional DC maturation stimuli such as CD40L or toll-like receptor ligands (TLR-L) to induce potent antitumor immune responses. Alternatively, DC-targeting motifs that simultaneously effect maturation and opening the possibility to use it for both priming and boosting (21). Unfortunately, DCs are relatively resistant to infection with Ad5 because of their lack of expression of the primary Ad5 receptor, coxsackie adenovirus receptor (CAR; ref. 22). We

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previously showed that this limitation could be overcome by retargeting Ad5 to CD40 by using bispecific adaptor molecules that simultaneously neutralized the Ad5 fiber knob and antigenically bound CD40 on the DC surface (16, 23). This approach facilitated the induction of T-cell-mediated immune responses against nonself transgene products (17, 24) and protected mice from tumor growth through vaccination with \textit{ex vivo} modified DCs (17). However, the capacity of this approach to induce effective immune responses against poorly immunogenic TAAs by targeted delivery to DCs \textit{in vivo}, being its most promising application, has not yet been evaluated.

Melanoma antigens such as tyrosinase-related proteins 1 and 2 (TRP-1/-2) and gp100, are nonmutated autoantigens (25, 26) to which T-cell tolerance prevails (27), eliciting only low-avidity CD8$^+$ T cells because of thymic or extrathymic deletion of high-avidity autoreactive T-cell clones (28). Targeted \textit{in vivo} delivery of TAAs to DCs may break this tolerance (5). Moreover, agonistic binding of CD40 has been pinpointed as a vital mode of DC maturation to facilitate the induction of TAA-specific CTLs (29), thus providing a clear rationale for CD40-mediated DC targeting of Ad-based tumor vaccines. Here, we show that \textit{in vivo} delivery through the skin of a CD40-targeted Ad5 vector leads to selective \textit{in vivo} transduction of DCs in the skin and injection site–draining lymph nodes, resulting in increased CD8$^+$ T-cell responsiveness and enhanced therapeutic efficacy in the murine B16F10 melanoma model.

**Materials and Methods**

**Animals and cell lines**

Five- to eight-week-old C57BL/6 mice (H-2K$^b$; Harlan Netherlands B.V.) were used for this study, which was approved by the Institutional Experimental Animal Ethics Committee. The murine melanoma cell line B16F10 (H-2K$^b$, kindly provided to the Institutional Experimental Animal Ethics Committee. The erlands B.V.) were used for this study, which was approved by 2Kb

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**Peptides**

The H-2K$^b$–binding peptides SVYDFFVWL (TRP2aa180–188) derived from the murine melanosomal protein TRP2), ICP- MYARV (Gal$^{galactic}$ derived from Escherichia coli β-galactosidase), and the H2-D$^b$–binding peptide KVPRNQDWL (gp100225–33) derived from the human melanosomal protein gp100 (30) were purchased from Leiden University Medical Center, The Netherlands.

**Ad vectors**

The following replication defective, E1-substituted and E3-deleted Ad5 vectors were used: (i) Ad-GFP, encoding enhanced green fluorescent protein (eGFP, ref. 31), (ii) Ad-gp100, encoding full-length human gp100 (32), and (iii) Ad-GFP-TRP2aa180–188, encoding the immunodominant H-2K$^b$–binding epitope TRP2aa180–188 fused to eGFP, under the control of the cytomegalovirus promoter (33). These Ad vectors were propagated, titrated, and stored as described previously (34).

**Recombinant Cfm40L adaptor protein**

A recombinant molecular adaptor protein consisting of the soluble ectodomain of CAR linked to the ectodomain of mouse CD40 ligand via a trimerization motif (Cfm40L) was constructed, produced, and purified as described previously (23). CD40-Ad-GFP-TRP2aa180–188, CD40-Ad-gp100, or CD40-Ad-GFP (i.e., CD40-targeted Ad) were prepared by preincubating 3.125 μg of Cfm40L with 60 × 10$^6$ viral particles (vp; 600 vp/cell) of Ad-GFP-TRP2aa180–188, Ad-gp100, or Ad-GFP for 30 minutes in 10 to 20 μL of serum-free medium at room temperature.

**Bone marrow–derived DCs, skin DCs, and Ad infections**

Bone marrow–derived DCs (BMDC) were generated as described previously by Samsom and colleagues (35) and were used for Ad infections. Murine ears were collected from sacrificed C57BL/6 mice and washed in 70% alcohol. The dorsal and ventral halves of the ears were separated mechanically and dorsal halves were cultured in 24-well plates in RPMI complete medium for 48 hours at 37°C. Migrated DCs were harvested and used for Ad infections as described below.

DCs were seeded at a density of 0.5 × 10$^6$ cells per well in a 24-well plate in 500 μL serum-free medium and were transduced with CD40-targeted or untargeted-Ad-GFP or Ad-GFP-TRP2aa180–188 at 37°C for 2 hours at a multiplicity of infection (MOI) of 600 based on vp. After 2 hours of incubation, 2 mL of serum containing IMDM was added. Transduced DCs were harvested after 48 hours, washed repeatedly, and used either for flow cytometric analysis or vaccination.

**Vaccination and vaccination site excision**

Mice were vaccinated as indicated with Ad-transduced BMDCs (0.5 × 10$^6$ per mouse), CD40-targeted- or untargeted-Ad [dose: 10 × 10$^6$ infectious units per mouse of Ad-GFP-TRP2aa180–188 (vp/infectious units = 51) or 1 × 10$^6$ infectious units/mouse/vaccination of Ad-gp100 (vp/infectious units = 31)]. Vaccines were suspended in sterile PBS in a total volume of 50 μL and were injected via the intradermal (i.d.) route on the flank with a MicroFine insulin syringe with a 29G needle (BD Biosciences). In some experiments, the vaccination site was excised 4 hours after i.d. vaccination and the excision wound was closed by using surgical staples and glue (3M Nederland BV).

**IFN-γ intracellular staining**

The IFN-γ intracellular staining assay was carried out to detect TRP2aa180–188 and gp100225–33 specific T cells in mouse
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Isolation and Ad transduction of murine lymph node cells and splenocytes

Lymph node and spleen cells were harvested from mice and single cell suspensions were made by chopping the tissues with scissors and gently pressing them through 100 μm filters (BD Biosciences). Finally, the lymph node and spleen cells were washed twice in serum-free medium, counted, and seeded at a density of 1 × 10^6 cells per well of 24-well plates in 500 μL of serum-free medium. Cells were infected with Ad-GFP or CD40-Ad-GFP at an MOI of 300 (vp, calculated on the basis of DC numbers) for 2 hours at 37°C. After infection, 2 mL of IMDM complete medium was added and the cells were cultured for 2 days. Subsequently, cells were harvested, washed, labeled with PE-labeled anti-MHC-II (eBioscience) and/or anti-B220, and/or APC-labeled anti-CD11c and appropriate isotype control antibodies (BD Biosciences), and subjected to flow cytometric analysis.

In vitro antigen presentation assay

BMDCs or DCs in lymph node suspensions were transduced with CD40-targeted- or untargeted-Ad vectors encoding GFP or TRP2aa180–188 fused to GFP. Transgene-expressing DCs were plated in round-bottom 96-well plates (Greiner Bio-one) with splenocytes containing previously primed TRP2aa180–188-specific CD8^+ T cells, isolated from mice vaccinated with Ad-GFP-TRP2aa180–188, at a ratio of 1:10. Splenocytes stimulated with DCs loaded with TRP2aa180–188 or βgalaa497–504 peptides were used as controls. After overnight stimulation, the percentage of CD8^+ T cells producing intracellular IFN-γ was determined by flow cytometry as described previously (34).

Imaging and quantification of transgene expression in vivo

Transgenic GFP expression levels at the site of vaccination and in vaccination site–draining lymph nodes were measured 24 hours after injection of Ad vectors, using an IVIS Lumina II Bioluminescent Imager (Xenogen). Images and measurements of transgene expression were captured for 10 seconds and analyzed with Living Image software (Xenogen). The GFP expression was imaged and digitally displayed as a pseudocolor overlay on the gray-scale image of the animal or excised lymph nodes. Regions of interest were drawn around the transgene expression site digitally on the displayed images and quantified as photons/sec/cm^2/sr.

Tumor inoculation and measurements

Syngeneic B16F10 cells (3 × 10^5 suspended in 50 μL PBS) were implanted s.c. on the flank. Twice a week, 2 bisecting diameters of each tumor were measured with a digital slide caliper until the end of the experiment. The reported tumor volumes were calculated according to the formula \(0.4 \times (ab^2)\), with "a" as the larger and "b" as the smaller diameter (36). When tumors reached the maximal allowable volume according to Dutch national regulations, mice were sacrificed.

Tissue fixation and immunofluorescence microscopy

Skin biopsies from the site of vaccination and the vaccination site–draining lymph nodes [axillary, identified by injection of Evans Blue dye (Sigma)] were fixed in 4% paraformaldehyde and 10% sucrose in PBS at 4°C for 30 minutes (37). Fixed tissues were embedded in Tissue-Tec OCT (Sakura Finetek) above liquid nitrogen. Cryosections of 10 μm were cut from frozen tissue blocks and mounted on poly-L-lysine–coated slides. Slides were dried at room temperature overnight, fixed in acetone at 4°C for 10 minutes, and then stored at −20°C until use.

Slides were brought to room temperature and placed in PBS for 5 minutes to remove OCT; endogenous biotin was blocked (DAKO Corporation) and slides were stained with biotinylated anti-IA/IE or anti-CD11c mAbs (BD Pharmin- gen). Specific binding of the antibody was detected with Cy3 conjugated streptavidin (Invitrogen), 4′,6-Diamidino-2-phenylindole (DAPI; Molecular probes) was used to stain nuclei and slides were mounted with mounting medium (Immunoconcepts NA Ltd). Staining was evaluated by fluorescence microscopy (Nikon instruments BV) with appropriate filters.

Statistical analysis

Data were analyzed by 1-way ANOVA with Bonferroni correction using GraphPad Prism software.

Results

Enhanced in vitro transduction and maturation of BMDCs by CD40-Ad and enhanced prophylactic vaccination efficacy

BMDCs were more efficiently transduced by CD40-Ad than by untargeted-Ad as determined by GFP transgene expression (Fig. 1A) and simultaneously underwent maturation as determined by expression levels of the DC maturation markers MHC-II and CD86 (Fig. 1B). To compare the antitumor efficacy of CD40-Ad and untargeted-Ad, we used the aggressive and poorly immunogenic murine B16F10 melanoma model (38). Mice were vaccinated with syngeneic BMDCs transduced in vitro by CD40-targeted- or untargeted-Ad-GFP-TRP2aa180–188. Animals were subsequently inoculated s.c. with B16F10 cells on the distant flank (see Fig. 1C for a schedule of the experiment). BMDCs transduced by CD40-Ad-GFP-TRP2aa180–188 exhibited superior vaccination efficacy over BMDCs transduced with Ad-GFP-TRP2aa180–188 (Fig. 1D and E) and induced stronger TRP2aa180–188-specific CD8^+ T-cell responses in peripheral blood (Fig. 1D).

Enhanced DC transduction and MHC-I–mediated presentation of transgenic antigens through CD40-targeted transduction of DCs in lymph node suspensions

To ascertain the ability of CD40-Ad to target and transduce tissue-derived DCs, we carried out in vitro experiments with skin-derived DCs as well as with lymph node and spleen...
suspensions. Although transduction levels were low, CD40-mediated Ad infection significantly improved the transduction efficiency of CD11c+ DCs migrated from murine ear skin explants (Fig. 2A). Because CD40 expression is not exclusively restricted to DCs, we evaluated the tropism of CD40-Ad in single-cell suspensions of murine lymph nodes and spleen. CD40-Ad very efficiently and selectively transduced DCs, identified as CD11c and MHC-II double positive cells, but not B220+B cells, in both lymph nodes and spleen single-cell suspensions (Fig. 2A).

To test whether DCs in lymph node suspensions, transduced by CD40-Ad, presented endogenously synthesized and processed transgene-encoded antigens more efficiently than untargeted-Ad-transduced DCs, we assessed the in vitro antigen presentation efficiency of lymph node samples transduced either by CD40-targeted- or untargeted-Ad-GFP-TRP2aa180–188. Data presented in Fig. 2B show that lymph node DC transduction with a CD40-Ad vector encoding TRP2aa180–188 and GFP led to the activation of higher rates of specific IFN-γ-producing CD8+ T cells in TRP2aa180–188-primed splenocytes than transduction with the untargeted vector, although this did not reach significance, likely because of background reactivity, for example, against GFP.

**CD40 targeting of Ads in vivo dramatically reduces total transgene expression levels but results in more selective DC transduction**

Retargeting Ads to CD40 may reduce undue toxicity of Ads by blocking the naturally broad tropism of Ad vectors. To this end, we assessed the in vivo GFP transgene expression at the site of vaccination and in excised draining lymph nodes, using bioluminescent imaging. Total transgene expression levels were significantly lower at the site of vaccination in mice injected with CD40-Ad compared with untargeted-Ad 24 hours after injection (Fig. 3A and B). Immunohistochemical and fluorescence microscopic examination revealed sparse transduction of DCs at the skin injection site (Fig. 3C). Comparable reduction in total transgene expression levels (Fig. 4A and B), but more selective transduction of DC, was also observed in the vaccination site–draining lymph nodes (Fig. 4C).
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Figure 2. Targeting Ad to CD40 selectively enhances the transduction of skin, lymph nodes, and splenic DCs and improves MHC-I-mediated presentation of transgenic antigens. A, percentages of CD11c+ skin-emigrated DCs, and MHC-II+ and CD11c+ DCs and B220+ B cells in lymph node and spleen single-cell suspensions, expressing GFP after in vitro transduction with Ad-GFP or CD40-Ad-GFP. B, Lymph node DCs (LNDc) were transduced in vitro with Ad-GFP-TRP2aa180 or CD40-Ad-GFP-TRP2aa180 and used to stimulate splenocytes from Ad-GFP-TRP2aa180-immunized mice to compare antigen presentation ability. Lymph node DCs pulsed with either TRP2aa180 or β-galaa497-504 were included as positive and negative control, respectively. The percentage of IFN-γ-producing cells within CD8+ cells was determined by flow cytometry after intracellular staining. Mean ± SEM are shown from 3 independent experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

indicates a drastic reduction in the number of transduced cells resulting from an effective block of the natural tropism of the Ad vector by the CFm40L retargeting adaptor protein. In contrast, injection of untargeted-Ad led to massive transduction of larger cells, both in skin and in lymph nodes, which, by their morphologic features, their lack of CD11c expression, and their localization, most likely represented fibroblasts and/or macrophages (Supplementary Fig. S1A). Note that assessment of colocalization of MHC class II and low-level GFP expression in the skin (as a measure of DC transduction) was further complicated by autofluorescence of hair follicles and their close proximity to surrounding DCs (exemplified in Supplementary Fig. S1B; hair follicles indicated by asterisks in Fig. 3). Although rare and at low levels, and thus hard to show immunohistochemically, the targeted transduction of cutaneous DCs by CD40-Ad was supported by surgical removal of the dermal vaccination site 4 hours after vaccination with CD40-targeted or untargeted-Ad-GFP-TRP2aa180-188. This surgical intervention almost completely abrogated the priming of TRP2aa180–188-specific CD8+ T cells (Supplementary Fig. S2A) as well as antitumor efficacy (Supplementary Fig. S2B) in mice vaccinated with CD40-Ad-GFP-TRP2aa180–188 but not in mice vaccinated with untargeted-Ad-GFP-TRP2aa180–188. This indicates rapid and selective transduction of cutaneous DCs by CD40-targeted Ad vectors, followed by their migration to draining lymph nodes to prime an effective antitumor response. In contrast, untargeted-Ad vectors may depend more on later cross-priming events and/or transduction of DCs in the lymph nodes for their antitumor efficacy.

Intradermally delivered untargeted-Ad vectors preferentially transduced large cells in the marginal lymph node sinuses, most likely representing macrophages (Supplementary Fig. S1A). To confirm the uptake of untargeted-Ad vectors by macrophages, regional macrophages were depleted by injection of clodronate-containing liposomes prior to vaccination of mice with the CD40-targeted- or untargeted-Ad-GFP-TRP2aa180-188 vector. Specific T-cell responses in peripheral blood were significantly increased by local depletion of macrophages for the untargeted, but not the CD40-targeted vector (Supplementary Fig. S2C). This indicates that CD40 targeting of Ad prevents scavenging by macrophages that might otherwise interfere with subsequent T-cell activation. Nevertheless, even in the presence of Ad-binding macrophages, small numbers of DCs may be transduced by untargeted Ad and thus effect direct CTL priming.

CD40-targeted intradermal delivery significantly improves the antitumor efficacy of an Ad5 vector encoding a full-length weakly immunogenic tumor antigen

We next tested the antitumor efficacy of i.d. injected Ad vaccines. Direct in vivo delivery of untargeted-Ad-GFP-TRP2aa180–188 induced potent TRP2aa180–188-specific CD8+ T-cell responses and conferred prophylactic protection against tumor outgrowth, neither of which was improved by CD40 retargeting of the employed Ad5 vector (Fig. 5A–C). These results indicate that i.d. delivered Ad vectors encoding an epitope of a self antigen linked to an immunogenic nonself protein (in this case GFP) can break tolerance for this epitope and induce potent cell-mediated antitumor immunity, relying on cross-priming without further requirement of redirected targeting to professional antigen-presenting cells (APC). Of
note, this was further supported by the observation that the antitumor efficacy of the i.d. delivered untargeted-Ad vector was superior to that afforded by \textit{ex vivo} Ad-transduced and i.d. injected BMDCs (Fig. 1D). To assess the effect of CD40 targeting on the induction of immunity against weakly immunogenic TAA, subsequent experiments (see Fig. 6A for experimental schedule) were conducted with an Ad vector encoding a full-length TAA of clinical relevance, that is, gp100. Importantly, CD40 targeting of Ad-gp100 significantly enhanced the induction of a gp100\textsubscript{25–33} specific CD8\textsuperscript{+} T-cell response (Fig. 6B) and accordingly also enhanced the antitumor efficacy, both prophylactically (not shown) and, more importantly, therapeutically (Fig. 6C). This also translated into an improved survival of tumor-bearing animals receiving a CD40-Ad-gp100 vaccine (Fig. 6D). These results thus clearly show the enhanced antitumor efficacy afforded by CD40 mediated \textit{in vivo} targeting to DCs of Ad vaccines encoding weakly immunogenic full-length TAAs.

\section*{Discussion}

Direct \textit{in vivo} delivery of full-length melanoma antigen genes such as TRP2 or gp100 offers a standardized off-the-shelf vaccination approach but does not induce protective immunity even with strongly immunogenic viral vehicles (30, 39–42). We therefore investigated the suitability of CD40-targeted Ad vectors to selectively deliver open reading frames encoding a TRP2-derived epitope or full-length gp100 to DC \textit{in vivo}, and evaluated their therapeutic efficacy in the murine B16F10 melanoma model. Our results show that the direct \textit{in vivo} administration of CD40-Ad significantly blocks the otherwise predominant transduction of non-APC and leads to more selective transduction of CD11c\textsuperscript{+} DCs in skin and draining lymph nodes. Consequently, a significantly improved therapeutic antitumor efficacy was observed for an i.d. delivered Ad-gp100 vaccine through CD40 targeting.
The efficacy of *ex vivo* loaded DCs, which are often used as cancer vaccines (43), may be improved upon by transduction with CD40-Ad (17, 44). This approach is however wrought with issues about logistics and consistency; *in vivo* targeting of antigens to DCs would instead allow for a more standardized vaccination method (5). Targeting proteins or antigenic peptides to cutaneous DCs *in vivo* through their conjugation to antibodies against, for example, DEC-205, DCIR2, or MHC-II, has led to T-cell anergy and the induction of T-regulatory cells (Treg), unless CpGs or agonistic anti-CD40s were admixed to ensure long-term memory and effective antitumor responses (8, 45). The CD40 receptor represents an attractive DC-targeting motif in itself, as it plays an important role in the induction of DC maturation and priming of cytotoxic T cells (46). Indeed, we previously reported the selective *in situ* transduction of DCs and the simultaneous enhancement of their (tumor) antigen-specific CD8+ T-cell stimulatory capacity by CD40-Ad in a human skin explant model (15) and in human melanoma-draining lymph node suspensions (47). Such demonstrations of selective DC transduction in more complex tissue micro-environments are important because CD40 expression is not exclusively restricted to DCs but is also found in macrophages, B cells, and epithelial cells, which are poorly equipped to prime an effective CTL response. DCs in single-cell suspensions of murine lymph nodes and spleen were also selectively and more efficiently transduced by CD40-Ad than by untargeted-Ad, and displayed enhanced presentation of the transferred gene products in the context of MHC class I. Similarly, CD46- or CD80/CD86-targeted Ad vectors have been reported to selectively transduce DC despite high expression levels of these receptors on, for example, monocytes and B cells (48). These differences in infection susceptibility may at least in part be attributed to the presence or absence of active endocytic pathways (49).

*Our in vivo* bioluminescent imaging data clearly showed that *in vivo* retargeting of Ad to CD40 almost completely ablated its natural tropism in both murine skin and
skin-draining lymph nodes. Retargeting may thus reduce unwanted cytopathic side effects associated with the clinical use of Ad vectors. This reduction in overall transgenic antigen load was accompanied by a more selective delivery of the transgene to CD11c⁺ DCs in both skin and draining lymph nodes. Moreover, removal of the skin vaccination site after 4 hours effectively blocked the induction of specific CD8⁺ T cells and reduced associated antitumor efficacy, implying a vital role for cutaneous DC transduction by CD40-Ad in this regard.

We observed a consistent association between the level of CD8⁺ T-cell activation and antitumor efficacy. Indeed, previous studies with CD40-Ad-transduced DC vaccines showed a complete dependence of the observed antitumor efficacy on CD8⁺ T cells and less so on CD4⁺ T cells, presumably because of the CD40-mediated activation of the transduced DCs, which might have substituted in part for the natural role of T-helper (Th) cells in this regard. Consistent with this notion, CD40-targeted dermal delivery of full-length human gp100, which is 80% homologous to murine gp100 at the amino acid level (42), afforded a clear advantage both in terms of CD8⁺ T-cell responses and confers protection against B16F10 tumor growth, neither of which is improved upon by CD40 retargeting.

In summary, the present data show that CD40-targeted Ad vectors can be used to efficiently and selectively deliver TAAs to DCs in vivo and to enhance their TAA-specific CD8⁺ T-cell stimulatory capacity. Most importantly, CD40 targeting significantly improved the therapeutic antitumor efficacy of an Ad vector encoding full-length gp100 in the B16F10 melanoma model. Combined with our previous observations in human skin explant and lymph node models, these data make a strong case for the clinical development of CD40-Ad vectors for the in...
vivo targeting and transduction of cutaneous DCs for cancer immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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