PD-1 or PD-L1 Blockade Restores Antitumor Efficacy Following SSX2 Epitope-Modified DNA Vaccine Immunization

Brian T. Rekoske¹, Heath A. Smith², Brian M. Olson³, Brett B. Maricque³, and Douglas G. McNeel¹,³

Abstract

DNA vaccines have demonstrated antitumor efficacy in multiple preclinical models, but low immunogenicity has been observed in several human clinical trials. This has led to many approaches seeking to improve the immunogenicity of DNA vaccines. We previously reported that a DNA vaccine encoding the cancer-testis antigen SSX2, modified to encode altered epitopes with increased MHC class I affinity, elicited a greater frequency of cytolytic, multifunctional CD8+ T cells in non-tumor-bearing mice. We sought to test whether this optimized vaccine resulted in increased antitumor activity in mice bearing an HLA-A2–expressing tumor engineered to express SSX2. We found that immunization of tumor-bearing mice with the optimized vaccine elicited a surprisingly inferior antitumor effect relative to the native vaccine. Both native and optimized vaccines led to increased expression of PD-L1 on tumor cells, but antigen-specific CD8+ T cells from mice immunized with the optimized construct expressed higher PD-1. Splenocytes from immunized animals induced PD-L1 expression on tumor cells in vitro. Antitumor activity of the optimized vaccine could be increased when combined with antibodies blocking PD-1 or PD-L1, or by targeting a tumor line not expressing PD-L1. These findings suggest that vaccines aimed at eliciting effector CD8+ T cells, and DNA vaccines in particular, might best be combined with PD-1 pathway inhibitors in clinical trials. This strategy may be particularly advantageous for vaccines targeting prostate cancer, a disease for which antitumor vaccines have demonstrated clinical benefit and yet PD-1 pathway inhibitors alone have shown little efficacy to date. Cancer Immunol Res; 3(8): 946–55. ©2015 AACR.

Introduction

Prostate cancer is the most commonly diagnosed cancer in the United States and the second leading cause of cancer-related death in American men (1). Despite primary therapy with prostatectomy and/or radiotherapy, approximately one of three tumors will recur and can ultimately develop into castration-resistant metastatic disease; the lethal form of prostate cancer (2, 3). In 2010, sipuleucel-T (Provenge; Dendreon Corp.), an autologous cellular vaccine targeting prostatic acid phosphatase (PAP), was approved by the FDA for the treatment of patients with metastatic prostate cancer based on trials demonstrating an improved overall survival following treatment, underscoring the potential for antigen-specific vaccines to affect the clinical care of patients with advanced prostate cancer (4). Similarly, encouraging results observed in randomized phase II trials using PROSTVAC (rilimogene galvacirepvec/glafolevec, Bavarian Nordic), a viral-based vaccine targeting prostate-specific antigen (PSA), has renewed interest in the development of other antigen-specific immunotherapies for the treatment of prostate cancer and other malignancies (5). In fact, due to these and many other recent successes in the cancer immunotherapy field, including clinical results observed from T-cell checkpoint molecule blockade (PD-1, CTLA-4, etc.), the journal Science named cancer immunotherapy as its 'Breakthrough of the Year' for 2013 (6).

We have focused on DNA vaccines as an approach for the treatment of patients with recurrent prostate cancer. We have completed clinical trials evaluating the safety and administration schedule of a DNA vaccine encoding PAP, and a randomized phase II trial is currently ongoing (7, 8). However, despite being shown to be safe across many phase I clinical trials, and despite demonstrable efficacy as a treatment for diseases in other animals (including dogs, horses, and fish), no other human DNA vaccines for the treatment of cancer have progressed beyond phase I trials (9–12). As such, much effort has been devoted to better understanding of the mechanisms of action of DNA vaccines and exploring methods to enhance their immunogenicity and possible clinical effectiveness.

One such method that has been extensively studied is the encoding of altered peptide ligands (APL) with point mutations in the presented epitopes to enhance their binding affinity for the major histocompatibility complex (MHC) and/or the T-cell receptor (TCR; refs. 13, 14). These types of modifications have been shown to increase the immunogenicity of both peptide

¹Department of Medicine, University of Wisconsin–Madison, Madison, Wisconsin. ²Department of Oncology, University of Wisconsin–Madison, Madison, Wisconsin. ³The Carbone Cancer Center, University of Wisconsin–Madison, Madison, Wisconsin.

Note: Supplementary data for this article are available at Cancer Immunology Research Online (http://cancerimmunolres.aacrjournals.org/).

Corresponding Author: Douglas G. McNeel, University of Wisconsin–Madison, 7007 Wisconsin Institutes for Medical Research, 1111 Highland Avenue, Madison, WI 53705. Phone: 608-265-8131; Fax: 608-265-0614; E-mail: dms5@medicine.wisc.edu
doi: 10.1158/2326-6066.CIR-14-0206
©2015 American Association for Cancer Research.
and DNA vaccines targeting different viral and tumor antigens that were otherwise weakly immunogenic (15–18). One vaccine encoding an APL currently in clinical trials is PROSTVAC; the vaccinia- and fowlpox-based vaccine encoding PSA described above (19). In preclinical development of this vaccine, it was observed that a native HLA-A2-restricted PSA epitope was weakly immunogenic and that its immunogenicity could be enhanced when encoding an APL with enhanced MHC binding affinity (20, 21). We have studied synovial sarcoma X breakpoint 2 (SSX2) as a prostate tumor antigen, and have demonstrated that a DNA vaccine encoding SSX2 was able to elicit HLA-A2–restricted CD8+ T cells with cytolytic activity (22, 23). Recently, we identified that point mutations made to these epitopes could be used to immunize HLA-A2–expressing mice to elicit higher frequency of CD8+ T cells that recognized the native epitopes (24). Furthermore, a DNA vaccine encoding these optimized epitopes (pTVG-SSX2opt) was able to elicit a greater frequency of antigen-specific multifunctional CD8+ T cells that were better able to lyse both peptide-pulsed target cells and the SSX2-expressing LNCaP prostate cancer cell line in vitro.

In the current study, we examined the in vivo antitumor efficacy of this optimized DNA vaccine using a novel murine syngeneic tumor cell line model developed in HLA-A2–expressing mice. We found that the optimized DNA vaccine elicited an inferior antitumor response relative to the native vaccine not encoding the APLs. We demonstrated that this inferior response was associated with increased expression of the immunoregulatory molecule programmed cell death-1 (PD-1) on antigen-specific CD8+ T cells elicited by the optimized vaccine, and that the inferior antitumor response could be rescued by using PD-1 or PD-L1–blocking antibodies in combination with vaccination, or by targeting a PD-L1–deficient tumor. These findings demonstrate that efforts to improve vaccine efficacy by encoding altered peptide ligands might actually have a deleterious effect by leading to higher PD-1 expression. Moreover, these findings suggest that combining PD-1 pathway blockade with vaccines, and DNA vaccines in particular to augment antigen-specific CD8+ T cells, is a rational approach for future human clinical trials.

Materials and Methods

Mice and cell lines

HLA-A2.01/HLA-DR1–expressing, murine MHC class I/II knockout mice (HHDII-DR1) on a C57Bl/6 background were obtained from Charles River Labs courtesy of Dr. François Lemonnier (Institut Pasteur, Paris, France; ref. 25). Mice were maintained under aseptic conditions and all experiments were conducted under an IACUC-approved protocol.

To generate the A2/Sarcoma cell line, HHDII-DR1 mice were injected subcutaneously with 0.5 mg 3-methylcholanthrene (Sigma-Aldrich). Once tumors became palpable, tumors were collected, minced, and cultured in DMEM/high glucose medium (Mediatech). A2/Sarcoma cells were then stably transduced with lentiviral constructs encoding either GFP or SSX2. Expression was confirmed by immunoblotting using an SSX2-specific monoclonal antibody (Clone 1A4; Abnova) or for GFP and MHC expression by flow cytometry.

To generate the A2/Sarcoma-SSX2-APD-L1 line, we utilized the CRISPR-Cas genomic editing system by transfecting the A2/Sarcoma-SSX2 cells with a CRISPR-U6-gRNA/CMB-Cas9-GFP plasmid (gRNA Sequence: TTTACTATCACGGCTCCAA; Sigma), and cells were sorted using a FACs Aria sorter for live/single/GFP+ cells. A line incapable of expressing PD-L1 in response to IFNγ was isolated, and genomic DNA from this line was collected and sequenced to confirm the PD-L1 deletion mutation (data not shown).

DNA constructs

DNA vaccines encoding native or modified SSX2 were purified and used as previously described (22, 24). pTVG-SSX2opt was previously described as pTVG-SSX2 p41-AL/p103-RF, and pTVG-SSX2X5 was previously described as pTVG-SSX2 p41-VP/p103-IP.

PD-1/PD-L1 antibodies

Antibodies for mouse PD-1 (G4) and PD-L1 (10B5), both gracious gifts from Dr. Lieping Chen (Yale University, New Haven, CT), were purified from Armenian Hamster hybridoma lines using a HiTrap Protein G column (GE Healthcare) following previously published methods (26–28).

DNA and peptide immunization studies

Six- to 8-week-old HHDII-DR1 mice were immunized with plasmid DNA or peptides as we have previously reported (22, 24). For tumor protection studies, HHDII-DR1 mice were immunized intradermally 6 times biweekly with 100 μg of native or modified SSX2 vaccines followed 2 weeks later by subcutaneous inoculation with 2 × 106 SSX2- or GFP-expressing sarcoma cells in contralateral flanks. Tumor-cell suspensions were prepared in 50% high concentration, LDEV-free Matrigel (BD Biosciences). Tumor volume was measured in cubic centimeters according to the formula: (p/6)(long axis)(short axis)2. For tumor therapy studies, animals were first inoculated with tumor cells followed by weekly vaccination beginning one day after tumor implantation. In tumor studies using PD-1/PD-L1–blocking antibodies, 100 μg of antibody (or IgG isotype control, BioLegend) was injected intraperitoneally on the day following each vaccination.

Flow cytometry analyses

Tumors obtained at necropsy were digested in media containing 1 mg/mL collagenase and 20 μg/mL DNase I (Sigma) for 2 hours at 37°C, and passed through a 100-μm screen to obtain a single-cell suspension. CD8+ T cells were isolated (STEMCELL Technologies) and then stained with anti-CD3 (17A2; eBioscience), anti-CD8 (G4; eBioscience) and anti-CD11b (M1/70; eBioscience), anti-GR1 (1A8; BD Bioscience), anti-CD4 (145-2C11; BD Bioscience), anti-4/50 (B6-120; Tonbo Biosciences), anti-CD11c (H36-23; BioLegend), and anti-CD49b (G15-7; BioLegend). For PD-1/PD-L1–blocking antibodies, 100 μg of antibody (or IgG isotype control, BioLegend) was injected intraperitoneally on the day following each vaccination.
immunization with the optimized vaccine elicited an identical, or even slightly better (albeit not statistically signiﬁcant) tumor response compared with animals receiving either control or optimized SSX2 vaccine. In comparison with the previous report in which the optimized vaccine was able to elicit T cells against HLA-A2* tumor cells in vitro (24). Others have shown that IFNγ can lead to upregulation of PD-L1 on tumor cells (29, 30), so we hypothesized that immunization with pTVG-SSX2opt might have elicited higher PD-L1 expression on tumors due to an increased number of antigen-speciﬁc IFNγ-secreting CD8+ T cells. As shown in Fig. 2, tumors from animals receiving either the native or optimized SSX2 vaccine had higher expression of PD-L1 than tumors from animals receiving the control vaccine, as demonstrated by low cytometry (Fig. 2A and B) or immunofluorescence staining (Fig. 2C). However, there was no statistically signiﬁcant difference in tumor PD-L1 expression between tumors from native and optimized vaccine-treated animals. This upregulation of PD-L1 was likely a result of IFNγ secretion by vaccine-elicited T cells, given that A2/Sarcoma cells were found to have increased expression of PD-L1 following in vitro culture with recombinant IFNγ (Fig. 2D). Moreover, this effect was demonstrated to be mediated by antigen-speciﬁc cells, given that culture of CD8+ T cells isolated from splenocytes from HHDII-DR1 mice immunized with pTVG-SSX2 versus control-immunized mice led to increased PD-L1 expression on SSX2-expressing tumor cells in vitro, but not on control tumor cells not expressing SSX2 (Fig. 2E).

Antitumor activity of the optimized SSX2 vaccine is recovered when targeting a tumor line not expressing PD-L1

To test whether the tumor expression of PD-L1 in response to vaccination was responsible for the observed inferior antitumor activity of the optimized SSX2 vaccine, we used the CRISPR/Cas genomic editing system to generate an A2/Sarcoma-SSX2 cell line incapable of expressing PD-L1 in response to IFNγ (Fig. 3A). This ΔPD-L1 tumor cell line was then implanted, as described before, and animals were immunized weekly with either the control, native, or optimized SSX2 vectors. As shown in Fig. 3B, immunization with the optimized vaccine elicited an identical, or even slightly better (albeit not statistically signiﬁcantly greater), antitumor response compared with the native vaccine. In comparison with the previous ﬁndings (Fig. 1B), this suggested that tumor PD-L1 expression was at least partially responsible for the inferior
antitumor response observed with the optimized vaccine. As shown in Fig. 3C, while the tumor cells did not express PD-L1 (as expected), both tumor-infiltrating myeloid-derived suppressor cells (MDSC) and macrophages expressed detectable levels of PD-L1, suggesting that even in these PD-L1–deficient tumors, the PD-1/PD-L1 regulatory axis might still be involved in repressing antitumor responses, as these tumors were not eradicated following immunization.

Antigen-specific CD8\(^+\) T cells express higher levels of PD-1 in animals that received the optimized SSX2 vaccine

Given that the expression of PD-L1 did not appear different between treatment groups receiving the native or optimized vaccine (Fig. 2), and yet the expression of PD-L1 was responsible for the decreased antitumor effect observed with the optimized vaccine (Fig. 3), we next assessed whether the expression of PD-1 on CD8\(^+\) TILs differed with respect to immunization. As shown (Fig. 4A), tumors from animals immunized as in Fig. 1B had detectable levels of CD8\(^+\) TILs, and these cells expressed detectable levels of PD-1. The expression of PD-1 on antigen-specific CD8\(^+\) T cells was specifically evaluated on peripheral CD8\(^+\) T cells elicited following vaccination of tumor-bearing mice with either the native or optimized plasmids. As shown in Fig. 4B, PD-1 expression was significantly higher on p41- and p103-tetramer\(^+\) CD8\(^+\) T cells following immunization with the optimized construct. This upregulation of PD-1 was found to be independent of the presence of tumor, as non–tumor-bearing HHDII-DR1 mice immunized with either the native or the optimized SSX2 DNA vaccines (or the corresponding native or optimized p41 and p103 peptides alone) demonstrated elevated PD-1 expression on antigen-specific CD8\(^+\) T cells (Fig. 4C). This finding, taken together with the observation that both native and optimized vaccines induced similar levels of PD-L1 expression on tumors, and the observation of increased antitumor activity of the optimized vaccine when targeting a ΔPD-L1 tumor, suggested that the increased expression of PD-1 was most likely responsible for the decreased antitumor effect following vaccination with the optimized vaccine. Presumably, ligation of PD-L1 expressed by tumors led to a decrease in effector function, as has been demonstrated by others (31).
Antibody blockade of PD-1 or PD-L1 increases the antitumor activity of DNA immunization

We had previously demonstrated that antigen-specific CD8+ T cells elicited by immunization with these APLs, or with DNA vaccines encoding these APLs, had greater cytolytic activity in vitro (24). However the HLA-A2+ tumor cell target lines used for these analyses (T2 and LNCaP) are human, and do not express PD-L1 following IFNγ stimulation (Supplementary Fig. S2). This observation, along with the findings shown in both Figs. 3 and 4, suggested that blocking the ligation of PD-1 on antigen-specific CD8+ T cells might restore or enhance the antitumor efficacy of the APL-encoding DNA vaccine, particularly given the persistence of PD-L1 expression in the tumor microenvironment shown in Fig. 3C. To test this possibility, mice were implanted with SSX2-expressing tumor cells, followed by weekly administration of either the control, native, or optimized SSX2 vaccines. Mice then received 100 μg of PD-1 or PD-L1 blocking antibody, or isotype control, 24 hours following vaccination. As before, the native vaccine elicited greater antitumor activity compared with pTVG-SSX2opt (Fig. 5). However, treatment with the combination of pTVG-SSX2opt and antibodies against either PD-1 or PD-L1 elicited similar antitumor activity to that of pTVG-SSX2, suggesting that the antitumor activity elicited by pTVG-SSX2opt could be rescued by blockade of either PD-1 or PD-L1. While not statistically different from controls, the frequency of CD8+ TILs was generally higher in animals receiving the native vaccine or
optimized vaccine with anti–PD-1, and the expression of PD-1 on activated CD8+ TILs was highest in animals receiving the combined treatment (Supplementary Fig. S3). Furthermore, we observed complete tumor eradication in several animals that received pTVG-SSX2opt in combination with anti–PD-1 antibody, suggesting that the combination therapy may be more effective than vaccination alone (Fig. 5A).

Discussion

Modifying vaccines to encode altered peptide ligands to enhance epitope binding to the MHC/TCR complex is a method that has been explored as a means of increasing the immunogenicity of vaccines targeting various tumor and viral antigens that are otherwise weakly immunogenic. We recently reported on one such epitope-modified vaccine, a DNA vaccine encoding SSX2, that was able to elicit a greater frequency of antigen-specific multifunctional T cells with greater in vitro cytolytic activity (24). In this report, we sought to identify whether this vaccine encoding modified epitopes was able to elicit a stronger antitumor response against an SSX2-expressing tumor cell line in vivo. We demonstrated that this modified vaccine elicited an inferior antitumor response relative to the native vaccine. We found that this was associated with increased PD-1 expression on antigen-specific CD8+ T cells elicited from the optimized vaccine relative to those elicited by the native vaccine, and that immunization with either construct upregulated expression of PD-L1 on antigen-expressing tumors. Finally, we found that the antitumor activity of the optimized vaccine could be increased either by targeting a tumor incapable of expressing PD-L1 or when combined with PD-1 pathway blockade. PD-1 blockade alone had no substantial antitumor activity.

Our results demonstrate that an attempt to enhance the antitumor efficacy of a DNA vaccine by encoding APLs was actually counterproductive, and this was due to increased PD-1 expression on the antigen-specific CD8+ T cells elicited. This is different from reports demonstrating that APLs can be an effective means to increase the efficacy of antitumor and antiviral vaccines, including one DNA vaccine (32, 33). However, it is unclear whether in these other models PD-1 was similarly upregulated. Moreover, the expression of PD-1 on CD8+ T cells may be of less relevance in circumstances in which the targets of these CD8+ T cells do not express a PD-1 ligand, conceivably this is the case in different tumor and viral antigen systems, and probably the reason we observed an enhanced cytolytic activity in vitro from splenocytes obtained from non–tumor-bearing animals immunized with this vaccine (24). Our findings, notably those shown in Fig. 4B, are consistent with a recent report demonstrating that T cells stimulated in vitro with peptides of varying affinity can lead to different levels of PD-1 expression (34). The precise relationship between epitope-binding affinity and PD-1 expression remains unknown and is a future direction of our research. However, these findings suggest that other methods could be explored to increase the efficacy of DNA vaccines. Smith and colleagues demonstrated that epitopes with slightly weakened binding affinity led to lower levels of PD-1 expression, suggesting that modifications to DNA vaccines that decrease epitope-binding affinity, while simultaneously increasing epitope presentation, might be an approach to limit PD-1 expression and increase the antitumor efficacy of DNA vaccines (34).

We also observed in this tumor model that PD-L1 expression levels increased following administration of an antigen-specific DNA vaccine. These findings are similar to those recently reported by Fu and colleagues in which administration of a cellular vaccine was found to upregulate PD-L1 expression on tumors following vaccination (30). In their study, the mechanism for this could not be precisely established due to the absence of a defined antigenic target. We found that this upregulation of PD-L1 was due, at least in part, to antigen-specific CD8+ T cells elicited with DNA vaccination, as PD-L1 upregulation could be replicated in vitro by culturing the tumor cell line expressing the antigen in the presence of antigen-specific CD8+ T cells from immunized animals.
Findings have implications for the broader tumor immunology field, suggesting that while antitumor vaccines have potential efficacy in augmenting tumor-specific cytolytic CD8⁺ T cells, they may concurrently augment counterproductive regulatory ligands present in the tumor microenvironment. This possibility is supported further by our findings that the optimized

Figure 4.
Antigen-specific CD8⁺ T cells express higher levels of PD-1 in animals that received the optimized SSX2 vaccine. A, FFPE tumor sections obtained at necropsy were stained for CD8 and PD-1 expression. Shown are both the whole tumor sections (×10, assembled using Nikon Elements software) and higher powered images (×20) from representative animals from each treatment group. For the assembled tumor sections, the scale bar in the top left image indicates a length of 500 μm, and the red boxes indicate the regions where the higher-power images were taken. B and C, CD8⁺ T cells were isolated from splenocytes from tumor-bearing (B) or tumor-free (C) HHDII-DR1 mice that had received the indicated peptide or DNA vaccine treatment, and stained for SSX2 p41 or p103 with tetramers (separated in B, pooled in C) and for PD-1 expression. Shown are the mean fluorescence intensity (MFI) values for PD-1 expression on individual live/CD8⁺/tetramer⁺ events, with the median represented by the solid gray line. Each test represents splenocytes from at least 3 animals per group pooled together; *, P < 0.05 using a Mann–Whitney test.
Antibody blockade of PD-1 or PD-L1 increases the antitumor activity of DNA immunization. HHDIIR1 animals (n = 4–6/group) were subcutaneously implanted with SSX2-expressing sarcoma cells followed by weekly immunizations with pTVG4, pTVG-SSX2, or pTVG-SSX2opt. On the day after each immunization, all animals were treated with 100 μg of antibodies blocking PD-1 or PD-L1, or with IgG isotype control (as indicated by the graphs). Animals were subsequently monitored for tumor growth. Shown are individual animal tumor growth curves (A) and group means with SE (B). Results are representative of three independent studies. On graphs in A, the number of animals with a complete tumor response (CR) is noted. Error bars, SE; *P < 0.05 by the Mann-Whitney test.

Figure 5.

DNA Vaccine Antitumor Efficacy Augmented with PD-1 Blockade

evaccine had an enhanced antitumor response when targeting tumors engineered to not express PD-L1. At this point, it is not known whether upregulation of the PD-1/PD-L1 axis is specific to genetic vaccines as the method of immunization. This possibility is suggested by the slightly higher, albeit not statistically significant higher, PD-1 expression observed on CD8+ T cells following DNA immunization compared with direct peptide immunization (Fig. 3B). Given that DNA vaccines elicit a Th1-biased response, and CD8+ T-cell responses in particular, it seems likely that this could be a major mechanism of regulation; however, more studies are needed to confirm this.

Our findings are also of potential relevance to the broader tumor immunology field, given the recent FDA approval of Keytruda (pembrolizumab; Merck), and OpDivov (nivolumab; Bristol-Myers Squibb), two anti-PD-1 monoclonal antibodies for the treatment of ipilimumab-refractory melanoma, and pembrolizumab for the treatment of non-small cell lung cancer. Several studies have focused on the identification of predictive biomarkers to identify patients likely to respond to anti-PD-1 monotherapy, and the best characterized is tumor expression of PD-L1 (35, 36). Our data are consistent with this observed phenomenon, namely that subjects with PD-L1 expression on tumor cells are likely those with preexisting populations of tumor antigen-specific CD8+ T cells that can secrete IFNγ in the tumor microenvironment, leading to PD-L1 upregulation. Patients without PD-L1 tumor expression, conversely, might not have sufficient populations of tumor antigen-specific CD8+ T cells. Thus, efforts to increase the frequency of tumor antigen-specific CD8+ T cells should be of key importance to increase the efficacy of agents targeting the PD-1/PD-L1 axis, and our results suggest this may be feasible by the use of tumor-targeted antigen-specific vaccines.

Our data are also potentially relevant to the tumor immunology field given a recent surge in attention toward developing personalized tumor antigen-specific vaccines based on the identification of mutated tumor-specific epitopes (37). This method generally proposes to sequence individual tumor exomes to identify tumor-specific, MHC-restricted epitopes based on the presence of mutations leading to novel epitopes. CD8+ T cells specific for such epitopes should escape thymic tolerance; however, our results suggest high-affinity T cells might similarly be regulated by the PD-1/PD ligand pathway. This could, in fact, help explain why checkpoint inhibition has been successful in tumor systems with higher frequencies of tumor-specific mutations (38).

Together, our findings provide a clear rationale for pursuing clinical trials combining cancer vaccines, and DNA vaccines in particular, with PD-1 blockade. In our model, we found that PD-1 blockade alone had little antitumor effect, but we observed a marked increase in antitumor activity when PD-1 blockade was combined with a DNA vaccine that is able to elicit tumor antigen-specific CD8+ T cells. Interestingly we also observed that the optimized vaccine elicited complete responses when combined with PD-1 blockade, but not when targeting APD-L1 tumors, suggesting that PD-L1 expression by both tumor cells and infiltrating immune cells plays a role in regulating antitumor immune responses. The role of these PD-L1-expressing hematopoietic cells in regulating antitumor immunity in the tumor microenvironment will be another area of future research. However, these findings are of particular relevance to the treatment of advanced, metastatic prostate cancer given that anti-PD-1 monotherapy has shown relatively little success in early-phase clinical trials (35, 39), whereas vaccines have already demonstrated clinical benefit (4, 5). This may be an ideal clinical setting in which to evaluate treatments combining antitumor vaccination to elicit tumor-specific CD8+ T cells in combination with PD-1 blockade.

Disclosure of Potential Conflicts of Interest

D.G. McNeel is a founder, consultant, reports receiving a commercial research grant, has ownership interest (including patents), and is a consultant/advisory board member for Madison Vaccines, Inc. No potential conflicts of interest were disclosed by the other authors.
Cancer Immunol Res; 3(8) August 2015

Author's Contributions
Conception and design: H.A. Smith, D.C. McNeel
Development of methodology: B.T. Rekoske, H.A. Smith, D.G. McNeel
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.T. Rekoske, H.A. Smith, B.M. Olson, B.B. Maricque
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.T. Rekoske, D.G. McNeel
Writing, review, and/or revision of the manuscript: B.T. Rekoske, H.A. Smith, B.B. Maricque, D.G. McNeel

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.T. Rekoske, D.G. McNeel
Study supervision: D.G. McNeel

Acknowledgments
The HLA-A2 transgenic HHDII-DR1 mice are the property of the Institut Pasteur, 25–28 rue du Docteur Roux, Paris, France 75015, and were provided by Dr. François Lemortier. The authors thank Dr. Lieping Chen for graciously providing them with the PD-1 and PD-L1 hybridoma lines, Drs. Nachmushu and Dhanalakshmi Chinnasamy for providing them with the GPD lentivirus, the NIH Tetramer Facility (Atlanta, GA) for tetramer reagents, the JWCCC Flow Cytometry core facility (and NIH small instrument grants 1S10RR025483-01 and 1S1000D01820-02), and Mr. Jordan Bloom, Dr. Laura Johnson, and Dr. Chris Zahn for technical assistance.

Grant Support
This work was supported by the U.S. Army Medical Research and Material Command Prostate Cancer Research Program (W81XWH-08-0-1341 and W81XWH-07-1-0038), by the NIH R01 CA142608 and NIRA T32 GM07215, and by the Prostate Cancer Foundation (2014 Movember Global Treatment Sciences Challenge Award).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 4, 2014; revised April 28, 2015; accepted May 17, 2015; published OnlineFirst June 3, 2015.

References

Downloaded from cancerimmunolres.aacrjournals.org on October 25, 2017. © 2015 American Association for Cancer Research.


Cancer Immunology Research

PD-1 or PD-L1 Blockade Restores Antitumor Efficacy Following SSX2 Epitope–Modified DNA Vaccine Immunization

Brian T. Rekoske, Heath A. Smith, Brian M. Olson, et al.


Updated version Access the most recent version of this article at:
doi:10.1158/2326-6066.CIR-14-0206

Supplementary Material Access the most recent supplemental material at:
http://cancerimmunolres.aacrjournals.org/content/suppl/2015/06/02/2326-6066.CIR-14-0206.DC1

Cited articles This article cites 39 articles, 18 of which you can access for free at:
http://cancerimmunolres.aacrjournals.org/content/3/8/946.full#ref-list-1

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cancerimmunolres.aacrjournals.org/content/3/8/946.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.