CD8⁺ T-cell Responses Rapidly Select for Antigen-Negative Tumor Cells in the Prostate

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Abstract

Stimulation of patients' immune systems for the treatment of solid tumors is an emerging therapeutic paradigm. The use of enriched autologous T cells for adoptive cell therapy or vaccination with antigen-loaded dendritic cells has shown clinical efficacy in melanoma and prostate cancer, respectively. However, the long-term effects of immune responses on selection and outgrowth of antigen-negative tumor cells in specific tumor types must be determined to understand and achieve long-term therapeutic effects. In this study, we have investigated the expression of a tumor-specific antigen in situ after treatment with tumor-specific CD8⁺ T cells in an autochthonous mouse model of prostate cancer. After T-cell treatment, aggregates of dead antigen-positive tumor cells were concentrated in the lumen of the prostate gland and were eventually eliminated from the prostate tissue. Despite the elimination of antigen-positive tumor cells, prostate tumor continued to grow in T-cell–treated mice. Interestingly, the remaining tumor cells were antigen negative and downregulated MHC class I expression. These results show that CD8⁺ T cells are effective in eliminating antigen-bearing prostate tumor cells but they also can select for the outgrowth of antigen-negative tumor cells. These findings provide insights into the requirements for an effective cancer immunotherapy within the prostate that not only induces potent immune responses but also avoids selection and outgrowth of antigen-negative tumor cells.

Introduction

A promising cancer immunotherapy is the induction of tumor antigen-specific CD8⁺ T-cell responses. An autologous dendritic cell vaccine targeting prostate acid phosphatase has recently been approved for the treatment of metastatic castration-resistant prostate cancer (1). Adoptive cell therapy (ACT), a treatment strategy that involves harvesting tumor-infiltrating T lymphocytes (TIL), expanding them ex vivo, and reintroducing the expanded T cells back into the patient (2), is being developed for the treatment of melanoma, in which strong tumor-associated antigens have been described (3). Despite its promise, one potential drawback of antigen-specific T-cell responses has been carried out in animal models. For example, the PA14 antigen (PA14) has been introduced into different tumors, including mastocytomas, plasmocytomas, and fibrosarcomas. When these tumors were transplanted into recipient mice followed with ACT with PA14-specific T cells, antigen loss and tumor outgrowth were observed in all tumor types (4). Similarly, when ovalbumin (OVA)-expressing B16 melanoma cells were transplanted into recipient mice followed with treatment with OVA-specific OT-1 CD8⁺ T cells, downregulation of OVA expression was also detected (5, 6). The downregulation of target antigen is not limited to model antigens that were overexpressed in tumor cells. Vaccination of mice transplanted with B16 melanomas induced CD8⁺ T-cell responses that selected for tumor cells with downregulated expression of tyrosinase and tyrosinase-related protein 2 antigens (7), suggesting that expression of endogenous tumor antigens is also subject to inhibition as a result of antitumor immune responses.

The ability of immune responses to sculpt the antigen repertoire of spontaneously arising tumors is less clear. In a genetically engineered model of melanoma, targeting the gp100 protein with tumor-specific T cells led to dedifferentiation of melanocytes with concurrent loss of the gp100 antigen (8). Another widely studied autochthonous tumor model is the transgenic adenocarcinoma of the mouse prostate (TRAMP) as a result of prostate-specific expression of the SV40 large T antigen (Tag). Numerous studies have examined the effect of immunotherapies on TRAMP tumor growth as a potential strategy for treating human prostate cancer. However, few studies have analyzed the effect of T cells on antigen expression within TRAMP tumors and the ability of T cells to select...
antigen-loss variants. A recent study examined the ability of anti-HA T cells to control the growth of prostate tumor in TRAMP mice engineered to express the HA antigen (9). Whereas tumor growth was assessed by prostate weight and histology, there was no examination of antigen expression in the prostate. Similarly, a telomerase vaccine was tested in the TRAMP model and shown to exhibit a protective effect, despite eventual tumor outgrowth (10). Whereas T-cell responses, prostate tumor histopathology, and total survival were measured, there was no measurement after vaccination of target-antigen expression (10). Additional studies evaluating the effect of anti-Tag T cells on TRAMP tumors have assessed tumor burden, but these studies have not assessed the levels of Tag protein in situ (11, 12). As such, a clearer understanding of immunoediting beyond transplantable tumor and melanoma models is needed.

In our study of CD8 T-cell–tumor cell interaction, we have introduced a β-gal-SIY transgene encoding a fusion protein of β-galactosidase with a nominal MHC class I epitope (SIYR-YGYL or SIY) recognized by the 2C clonotypic TCR onto the TRAMP mice (TRP-SIY; ref. 13). Adoptive transfer of naïve CD8 2C T cells into TRP-SIY mice followed by infection with influenza virus expressing the SIY epitope leads to activation and differentiation of transferred T cells into potent effector cells. As in human patients, effector T cells infiltrate into the prostate tumor tissue and are rapidly tolerated. Similarly to human TILs, the 2C T cells persist in the prostate tumor tissue (14) and express high levels of PD-1 (15). This system provides a tractable model to study in detail the effects of adoptively transferred T cells on tumor-specific antigen expression. Here, we show that infiltration of activated 2C T cells into the prostate leads to elimination of SIY-positive tumor cells. However, tumor in the prostate of 2C T cell-treated TRAMP model and shown to exhibit a protective effect, despite eventual tumor outgrowth (10). Whereas T-cell responses, prostate tumor histopathology, and total survival were measured, there was no measurement after vaccination of target-antigen expression (10). Additional studies evaluating the effect of anti-Tag T cells on TRAMP tumors have assessed tumor burden, but these studies have not assessed the levels of Tag protein in situ (11, 12). As such, a clearer understanding of immunoediting beyond transplantable tumor and melanoma models is needed.

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Materials and Methods

Mice, adoptive transfer, and influenza infection

TRP-SIY mice were generated as previously described (13). 2C and OT-1 TCR transgenic mice were maintained on C57BL/6 and RAG1−/− backgrounds. Where indicated, 16-week-old mice were retro-orbitally injected with 1.5 × 10⁶ naïve 2C or OT-1 cells from 2C/RAG or OT-1/RAG mice and immediately infected intranasally with 100 pfu WSN-SIY or WSN-SIIN virus, respectively. T-cell–treated and WSN-infected mice were paired with aged matched control mice where indicated. Experiments with mice were approved by the Committee on Animal Care at Massachusetts Institute of Technology (Cambridge, MA).

Immunohistochemistry

At indicated time points after T-cell treatment, the genitourinary tract was excised and all four prostate lobes dissected and flash frozen in optimum cutting temperature (OCT) compound (Tissue-Tek, Sakura). All tissues were sectioned at 10 μm thick by the Koch Institute Histology core facility along with matched hematoxylin and eosin (H&E) stained slides. For immunohistochemistry, frozen sections were acetone fixed and blocked with 0.3% H2O2 in PBS. Staining was conducted with Vectastain Elite ABC kit (Vector Laboratories) according to the manufacturer’s instructions. In some instances slides were stained with X-gal solution [5 mmol/L potassium ferri cyanide, 2 mmol/L magnesium chloride, 1 mg/mL X-gal stock solution (Promega) in PBS] for 3 hours before incubation with primary antibody. Biotinylated antibodies against Thy1.1 (clone HIS51, eBioscience), MHC class I (clone 28-8-6 BD Pharmingen), and CD31 (clone ME13.3, BioLegend) were visualized with DAB peroxidase staining kit (Vector Labs). Anti-SV40 large T antigen (clone Pab 101, BD Bioscience) staining was conducted with Vectastain Mouse on Mouse Peroxidase Kit (Vector Laboratories), according to the manufacturer’s instructions. Where indicated slides were counterstained with eosin (Sigma) or hematoxylin (Vector Labs).

Image and pathologic analysis

For image analysis, 10 random images were acquired for each sample representing all prostate glands on Zeiss Axiosplan II with ×10 or ×40 objective. For whole mount images, a MIRAX Midi (Carl Zeiss) slide scanner with Axiocam MR(m) camera (Carl Zeiss) was used and images captured using the MIRAX Viewer Software (Carl Zeiss). Peroxidase or β-gal staining was visualized on each image by thresholding and using preset protocols in Volocity 6.01 (PerkinElmer), such that a precise measurement of area could be made. The total area of the gland was determined on the basis of hematoxylin or eosin staining. Contact between stains as well as luminal and glandular measurements were individually outlined in Volocity and then measured using the preset protocols as described above. H&E-stained prostate lobes were blindly graded by Dr. Roderick Bronson at the Koch Institute for Integral Cancer Research Pathology Core Facility using the following scale: 0, normal tissue; 1, proliferation with no invasion; 2, early invasion; 3, clear-cut invasion; 4, total replacement of organ.

Statistical analysis

Data were analyzed with Prism 5.0 (GraphPad Software). Student t test was used for comparisons. A P value of less than 0.05 was considered statistically significant. In figures, * is indicative of P < 0.05; **, P < 0.01; and ***, P < 0.001. Bar graphs represent mean ± SD.

Results

Prostate-infiltrating T cells gradually lose contact with antigen-expressing tumor cells

To determine the localization of TILs relative to antigen-expressing cells in the prostate tumor tissue, we developed an immunohistochemical analysis for 2C T cells by staining for...
Thy1.1 (brown) and antigen (SIY)-expressing cancer cells by X-gal staining (blue) for β-galactosidase (β-gal) in the prostate tumor tissue. In agreement with previous flow cytometry analysis (13), Thy1.1+ 2C T cells were found in the prostate gland 11 days after transfer and infection (dpt; Fig. 1A). Eleven dpt was chosen as an initial time point because 2C T-cell infiltration into the TRP-SIY prostates starts 7 dpt and reaches the peak level around 10 dpt (13). To quantify the Thy1.1 signal, we determined the total area stained by Thy1.1 as a function of the total glandular area stained by eosin (Fig. 1B). Thy1.1 staining area (level) covered approximately 10% of the prostate tumor tissue at 11 dpt (Fig. 1B). However, by 35 to 50 dpt, the levels of 2C T-cell staining decreased by 75%. These results match the kinetics of T-cell levels in TRP-SIY prostates as quantified by flow cytometry analysis of dissociated tissues (13).

To determine whether 2C T cells were in contact with antigen-expressing tumor cells in the prostate tumor, Thy1.1+ staining was classified into five nonoverlapping categories based on relative distance from the antigen (β-gal) staining and localization in the prostate tissue: (i) contiguous staining of Thy1.1 and β-gal in the same gland (direct contact between 2C T cells and SIY-expressing tumor cells; Fig. 1C, center top); (ii) staining within antigen-positive glands but not in direct contact with antigen (Fig. 1C, top right); (iii) staining within antigen-negative glands (Fig. 1C, bottom left); (iv) staining outside of the glandular area in the interstitial space (Fig. 1C, bottom center); and (v) staining within the luminal space of the prostate gland (Fig. 1C, bottom right). We used this analysis to quantify the changes of 2C T cells within the prostate at 11, 35 and 50 dpt and expressed each category as a fraction of total Thy1.1 signal across the three time points (Fig. 1D). At 11 dpt, approximately

![Figure 1](https://example.com/figure1.png)
15% of 2C T cells were in direct contact with SIY-expressing cells, with the majority of cells residing within antigen-positive prostate glands. By 35 dpt only 5% of 2C T cells were in contact with SIY-expressing cells, although the majority still resided in the antigen-positive glands. After 50 dpt, however, very few 2C T cells were in contact with SIY-expressing cells or resided in the antigen-positive glands. Associated with the gradual loss of T-cell/antigen contact, the proportion of Thy1.1\(^+\) 2C T cells in the \(\beta\)-gal/C0 gland increased. These data show that over time tumor-infiltrating (2C) T cells gradually lose contact with antigen (SIY)-expressing tumor cells in the prostate tissue.

T-cell treatment leads to loss of antigen-expressing tumor cells in the prostate

To determine whether the decrease in T-cell/antigen contact within the prostate tissue was due to loss of antigen expression over time, we harvested prostate tissues from age-matched TRP-SIY mice with (treated) or without (untreated) 2C T-cell transfer/infection, stained tissue sections for \(\beta\)-gal, and quantified the percentage of \(\beta\)-gal staining as a function of total prostate glandular area. TRP-SIY prostates normally contain large areas of epithelia that stained positive for \(\beta\)-gal (Fig. 2A, left). Eleven days after 2C T-cell treatment, the percentage of \(\beta\)-gal–positive areas did not change significantly (Fig. 2A and B). However, by 35 and 50 dpt the level of \(\beta\)-gal (antigen) expression was reduced significantly. The loss of SIY-expressing cells was antigen specific as transfer of OT-1 T cells that recognize the SIINFEKL (SIIN) epitope and infection with WSN virus that expresses the SIIN epitope (WSN-SIIN) did not induce the loss of \(\beta\)-gal–expressing cells within the prostates of TRP-SIY mice (Fig. 2B).

We noticed the spatial distribution of X-gal staining was markedly different between untreated and 2C T-cell–treated mice at 11 dpt. In untreated mice, \(\beta\)-gal staining was spread throughout the prostate tissue (Fig. 2A, top), and more than 90% of staining was found in glandular epithelia (Fig. 2C). In contrast, in the treated mice approximately 80% of \(\beta\)-gal staining was found in the lumen of prostate glands. To determine whether the luminal \(\beta\)-gal–positive areas contained viable cells, we compared consecutive sections of tissue stained with either H&E or X-gal. Luminal areas that stained positive for \(\beta\)-gal after T-cell treatment were composed of hematoxylin (nuclear stain)-negative and eosin-positive (cytoskeleton) cellular debris (Fig. 2D). Furthermore, \(\beta\)-gal stains were also detected in the urethra of treated mice (data not shown). These data suggest that the loss of \(\beta\)-gal–expressing tumor cells is likely due to elimination by infiltrating antigen-specific 2C T cells and the mass of dead tumor cells are cleared through the luminal space.
**T-cell treatment leads to a decrease of MHC class I–positive areas in the prostate**

Immune responses within the tumor environment can affect antigen expression as well as molecules involved in antigen presentation, particularly MHC class I (16, 17). To determine whether 2C T-cell treatment of TRP-SIY mice leads to a decrease in the level of MHC I in prostate tissue, we stained prostate sections from treated and untreated TRP-SIY mice for MHC class I. Although there were some variations in the pattern and staining intensity in different glands of the same prostate tissue or among prostates of different mice, the overall MHC class I staining was primarily localized in the glands (Fig. 3A). We quantified MHC class I expression as a fraction of the total prostate tissue area by randomly selecting 10 prostate

![Image of Figure 3](https://example.com/figure3.png)

**Figure 3.** MHC class I-expressing areas are reduced in 2C T-cell–treated prostates. A and B, mice were treated as in Fig. 2 and the 2C T-cell–treated prostate tissues and age-matched untreated control tissues (untreated) were stained for MHC class I (dark brown) and hematoxylin (light brown) and quantified. Representative staining of MHC class I and hematoxylin from TRP-SIY prostate tissue at the indicated time point with or without treatment (A). The arrows point to MHC class I–positive staining in sections of 2C–treated mice at 35 and 50 dpt. Percentages of area within the prostate tissue staining positive for MHC class I in 2C T-cell–treated (triangles), untreated (circles), or TRP-SIY mice receiving OT-1 T cells activated with WSN-SIIN virus (diamonds) at indicated time points (B). Each symbol represents one mouse. C and D, prostate sections from age-matched untreated and 2C–treated mice were stained for CD31 and eosin, and the stained areas were quantified. Representative stains of CD31 (brown) and eosin (red) of untreated mice and treated mice 11, 35, and 50 dpt (C). Percentages (mean ± SD) of CD31+ areas in the prostate section of untreated (circles) and 2C–treated (triangles) mice at the indicated dpt (D). Scale bars in A and C, 100 μm. *, P < 0.05; ***, P < 0.001; n.s., not significant.
images per mouse and 3 to 5 mice per treatment (Fig. 3B). Eleven days after 2C T-cell treatment, the percentage of MHC class I+ tissue area in treated mice was higher than that of untreated mice (Fig. 3B). By 35 dpt, 2 of the 4 treated mice contained a similar percentage of MHC class I+ tissue area as untreated mice while the other 2 mice had much reduced percentage. By 50 dpt, only approximately 10% of prostate tissue exhibited MHC class I staining, as compared with 30% to 50% of MHC class I+ tissue in untreated mice (Fig. 3B; P < 0.001). The reduction of MHC class I+ tissue area was largely antigen specific, as the transfer of OT-1 T cells and the infection with WSN-SiN virus did not affect the expression of MHC class I within the prostates of TRP-SiY mice (Fig. 3B). In total, these data indicate that after long-term exposure to antigen-specific T cells, either cell surface expression of MHC class I or the percentage of MHC class I+ cells within the prostate epithelium is reduced.

In addition to antigen expression, activated T cells within tumors can disrupt tumor stroma, such as CD31+ endothelial cells (18). We determined the levels of tumor vasculature by staining prostate tissue for CD31. Prostate tissue in TRP-SiY mice was well vascularized with clear CD31 staining in both 2C T-cell–treated and untreated mice (Fig. 3C). No difference in the percentage of area that stained positive for CD31 was detected in the untreated and treated mice (Fig. 3D). Together, these data show that 2C T-cell response in the prostate tissue leads to a reduction of MHC class I expression but not the tumor vasculature.

**2C T-cell treatment does not alter tumor progression**

We next determined whether reduction in antigen-expressing prostate cells in treated mice affected tumor progression. To assess tumor progression, H&E-stained prostate tissues from 2C-treated and untreated mice were blinded and graded for tumor stage (Fig. 4A). At 11 dpt the average tumor grade was the same (1.4) in both treated and age-matched control mice (Fig. 4A). At 35 dpt the average tumor grades in treated and age-matched controls were 2.2 and 2.5, respectively. At 50 dpt the average grade was 3.8 for treated mice versus 2.8 for untreated mice. These data suggest that despite clearance of SiY-expressing cells in the TRP-SiY prostate tumors, tumors continue to progress.

To determine why disease score was similar between treated and age-matched control mice across various time points, we evaluated the expression of the SiY and the oncogene driving tumor growth in TRP-SiY mice. TRP-SiY mice were constructed by introducing the β-gal-SiY fusion transgene into the TRAMP mice, which carry the SV40 large T antigen (Tag) transgene. Although both transgenics were driven by the same prostate-specific promoter, the two transgenes were introduced into the mouse genome independently. It is possible that activated 2C T cells eliminate SiY-expressing tumors while those that do not express the SiY antigen grow out after T-cell treatment. To test this possibility, we stained untreated TRP-SiY prostate sections for both β-gal and Tag. Although most β-gal and Tag staining overlapped, a significant fraction of prostate area was stained positive for Tag but negative for β-gal (Fig. 4B). Furthermore, to assess the expression of the Tag after 2C T-cell treatment we stained treated and untreated prostate sections for Tag (Fig. 4C). At all three time points (11, 35, and 50 dpt), the 2C T-cell treatment did not affect the percentage of prostate tissue that stained positive for Tag (Fig. 4D). However, the percentage of Tag-positive area increased over time following 2C treatment, correlating with tumor progression. Taken together, these data suggest that although 2C T-cell treatment is effective in eliminating SiY-positive tumor cells, the SiY-negative tumor cells continue to progress.

**Discussion**

The TRAMP model has been widely used to study prostate cancer immunotherapy. Numerous studies have induced CD8+ T-cell responses to TRAMP tumor, including clonotypic CD8+ T cells specific for Tag, HA, or SiY epitopes that were engineered to be expressed in the tumor cells, or endogenous CD8+ T cells following immunization with a telomerase vaccine. Although antigen-specific CD8+ T cells were potently induced in each case, the effect on tumor growth is minimal or transient, as the outgrowth of tumor cells eventually kills the TRAMP mice. The lack of long-lasting efficacy has been attributed to the rapid tolerization of tumor-infiltrating T cells by the immunosuppressive tumor environment within TRAMP mice (13, 19, 20). In the current study, by quantifying the level and distribution of responding CD8+ T cells and antigen-bearing tumor cells in the prostate, we show that CD8+ T cells are very effective in eliminating antigen-bearing tumor cells in the prostate. One week after infiltration into the prostate tumor tissue, most of the antigen-bearing tumor cells are already eliminated. Notably, aggregates of dead antigen-positive tumor cells are found in the lumen of the prostate gland and in the urethra, suggesting that killed tumor cells are removed from the prostate tissue through the lumen and urethra. These findings suggest that CD8+ T cells are more effective than previously realized in eliminating antigen-positive tumor cells in the prostate and alternative mechanisms underlying the lack of efficacy on tumor progression and survival of TRAMP mice as observed in many studies.

Our observation of the selection and outgrowth of antigen-negative tumor cells in TRP-SiY mice provides such a mechanism. We show that while SiY+ tumor cells are eliminated soon after T-cell infiltration into the prostate, SiY- but Tag+ cells continue to grow within the tumor bed. The presence of Tag+ SiY+ (β-galactosidase) tumor cells in TRP-SiY mice before T-cell treatment further suggests that the outgrowth of Tag+ SiY+ tumor cells compromised the efficacy of 2C T-cell treatment. This observation is likely an instance of immune editing, through which immune responses keep tumors under control but antigen-negative tumor cells eventually outgrow and escape immune surveillance. Our findings suggest that targeting multiple epitopes in poorly immunogenic tumors may increase the efficacy of immunotherapies. Indeed, in a B16-OVA model in which treatment of mice with OT-1 T cells was unable to control tumor growth (6), treatment with both OT-1 and the gp-100-specific Pmel-1 T cells prevented tumor growth (21). Furthermore, our work suggests that targeting of oncogenic drivers of tumor growth with immunotherapy could
be a more productive strategy. Previous attempts to use T cells that recognize Tag epitopes in the TRAMP model have provided mixed results with regard to reduction in tumor burden (11, 12). Nevertheless, other models have demonstrated the therapeutic benefit of focusing the immune response toward overexpressed oncogenes, including Tag (18). The efficacy of combination immunotherapies that target oncogene epitopes and nononcogene epitopes should be investigated in this and other models.

In addition to selection and outgrowth of antigen (SIY)-negative tumor cells in the prostate of TRP-SIY mice following 2C T-cell treatment, we found a significant decrease in the percentage of MHC class I⁺ area in the prostate of treated mice than in untreated mice at 50 dpt. The reduction in the percentage of MHC class I⁺ area could result from elimination of antigen-expressing tumor cells and/or downregulation of MHC class I expression on the remaining prostate tumor cells. Selection and outgrowth of antigen-negative tumor cells and the lack of effect of clonotypic T cells specific for an irrelevant antigen (OT-1 T cells) on SIY expression in the prostate (Fig. 2B) highlight the first possibility. The modest, yet statistically significant effect of OT-1 T cells on the percentage of MHC class I⁺ area (Fig. 3B) would support the second mechanism. Although further studies are needed to determine the relative contribution of the two mechanisms, the reduction of MHC class I⁺ tumor cells likely contributes to the decreased T-cell antigen contact at 35 and 50 dpt (Fig. 1D). The decrease in T-cell antigen contact could result from a decreased adhesion of...
T cells to tumor cells and/or stoichiometric issues due to the loss of antigen-positive tumor cells. Consistent with the first possibility, infiltrating 2C T cells are rapidly tolerized in the prostate tumor environment (13), which may decrease their ability to adhere to tumor cells. By 50 dpt, SIY-expressing tumor cells have been eliminated and the percentage of MHC class I area has decreased significantly (Figs. 2B and 3B), which likely reduces 2C T-cell/antigen contact due to the paucity of antigen-positive cells in the prostate.

In human melanoma, esophageal, and ovarian cancers MHC class I loss is associated with poor prognosis (17, 22, 23). Data from human prostate cancers are mixed with regard to MHC class I expression. A study assessing patient samples from varying tumor stages (Gleason stages 6–10) found MHC class I expression in all cases (24). A more recent study assessed components of the antigen-processing machinery in 59 primary prostate carcinoma and prostate carcinoma cell lines. HLA class I was retained in most tumor lesions, but the intracellular antigen-processing machinery was not detected in at least 21% of the tumors (16). Regardless, under steady state conditions MHC class I cell surface expression is retained in TRAMP tissues (24). The role of immunotherapies in MHC class I expression within prostates of human patients is of interest, as MHC class I loss can profoundly influence the efficacy of adaptive immune responses.

The long-term effect on antigen expression and antigen-loss variants in many immunotherapeutic modalities is unknown. Clinical trials evaluating ACT in patients with relapsing metastatic melanoma demonstrate the emergence of antigen-loss variants (25–27). However, whether selection for antigen-negative tumor cells also occurs in other tumor types following induction of a therapeutic immune response has yet to be determined. The effect of the U.S. Food and Drug Administration approved dendritic cell vaccination protocol on antigen expression in primary or relapsing prostate tumors has not been reported. Collectively, these studies will have important implications for the selection of tumor-specific antigens and suggest selection of multiple antigens, including those that drive tumor progression, are attractive targets for generating clinical benefit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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