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Effect of Dendritic Cells Injection After Radical Prostatectomy on Prostate Cancer in Mice

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Keywords: cell therapy | dendritic cells | prostate cancer | radical prostatectomy | tumor immunology

ABSTRACT

Background: To explore the therapeutic and preventive effect of dendritic cells injection combined with radical prostatectomy on prostate cancer in mice.

Methods: We extracted antigens from mouse prostate cancer cells RM-1 and cocultured them with dendritic cells to induce maturation. We constructed in situ carcinoma and subcutaneous tumor models of the mouse prostate. The efficacy of dendritic cell injection combined with radical prostatectomy was evaluated in the carcinoma in situ model, and the ability of dendritic cells to prevent prostate cancer was evaluated in the subcutaneous tumor model. Means of assessment included ultrasonography, flow cytometry analysis, and Elisa.

Results: Dendritic cell injection combined with radical prostatectomy effectively inhibited the growth of prostate carcinoma in situ in mice, as well as the growth of subcutaneous tumors of prostate cancer in mice. After dendritic cell injection, the levels of CD4 + T cells and Treg cells in the spleens of mice were significantly increased, and the levels of IL-2 and TNF-γ in the peripheral serum were significantly increased.

Conclusions: Injection of mature dendritic cells induced by mouse prostate cancer cell RM-1 antigen can inhibit the growth of prostate cancer. Radical prostatectomy combined with dendritic cell injection might be a potential treatment strategy for prostate cancer.

1 | Introduction

Prostate cancer is one of the most frequent malignant tumors in male urinary system [1]. Prostate cancer has the second-highest incidence and the fifth-highest mortality rate among male malignancies, which accounts for 6.8% of all cancer deaths and 14.1% of new deaths [2, 3]. The incidence of prostate cancer is increasing year by year, and this trend is more obvious in

Xiaoli Zhang, Weicong Sang, and Xiaoping Hong contributed equally to this study.

developing countries [4]. Currently, the main treatment methods for prostate cancer include surgery, radiation therapy, targeted endocrine therapy, and chemotherapy [5]. However, these methods are only effective for early prostate cancer. Current treatments for metastatic and castration-resistant prostate cancer (CRPC) are unsatisfactory [6].

Radical prostatectomy is still one of the most effective methods for localized and locally progressive prostate cancer [7]. However, 27%–53% of patients who received RP developed local recurrence or distant metastasis within 10 years after surgery, and 16%–35% of patients needed second-line treatment within 5 years after treatment [8]. Local recurrence or distant metastasis occurs in 20%–30% of patients with early prostate cancer after surgery or radiation therapy [9]. For patients with recurrent prostate cancer, endocrine therapy is mostly used clinically, but most of these patients will develop CRPC, and CRPC will eventually develop into metastatic CRPC (mCRPC) [10].

Dendritic cells (DC) are the most powerful professional antigen-presenting cells (APC) in the body, which can efficiently take up, process, and present antigens [11]. Mature DC can effectively activate initial T cells and play a key role in initiating, regulating, and maintaining the immune response, as well as in stimulating the initial immune response and inducing immune tolerance [12]. Neoantigens produced during tumorigenesis can be presented by tumor cells or antigen-presenting cells, in which dendritic cells play a major role [13]. More and more attention has been paid to the presentation of tumor antigen by DC in clinic. Sipuleucel-T, the first autologous cell immunotherapy drug approved by the US Food and Drug Administration, uses DC as the main effector cell for the treatment of asymptomatic or mild symptoms of mCRPC [14]. Sipuleucel-T increased overall survival with metastatic prostate cancer by 4.1 months (25.8 months in the placebo group and 21.7 months in the trial group); however, there was no significant difference in the time to tumor progression (3.7 months in the placebo group and 3.6 months in the trial group) [15].

In this study, we tried a new method of an early postoperative period of RP combined with DC to treat PCa in mice and discussed the effectiveness and necessity of early post-RP combined with DC therapy.

2 | Materials and Methods

2.1 | Reagents and Antibodies

Active Recombinant Mouse CSF-2/GM-CSF Protein (RP01206) and Active Recombinant Mouse IL-4 Protein (RP01161) were purchased from Abclonal. FITC anti-mouse CD80 (104705), PE anti-mouse CD40 (157505), APC anti-mouse CD86 (105011), APC/FireTM 750 anti-mouse-I-A/I-E (107651) and Pacific BlueTM anti-mouse CD11c (117321) were purchased from Biolegend (Beijing, China). CD45 (557659), CD3 (553061), CD4 (550954), CD25 (564368), CD8 (566985), Fxop3 (560408) were purchased from BD Biosciences (America). Mouse IFN-γ ELISA kit (ab282874) and Mouse IL-2 ELISA Kit (ab100706) were purchased from Abcam.

2.2 | Cell Culture

Mouse prostate cancer cell line RM-1 was purchased from the Cell Bank of the Chinese Academy of Science. RM-1 cells were cultured in RPMI 1640 medium (Gbico; Thermo Fisher Scientific Inc.) supplemented with 10% fetal bovine serum (FBS; Gbico; Thermo Fisher Scientific Inc.) and 1% P/S (Gbico; Thermo Fisher Scientific Inc.).

2.3 | BMDC Isolation and Induction

We isolated myelomonocytes from the femoral and tibial bone marrow of 8-week-old C57BL/6 mice. We euthanized the mice according to procedures approved by the Animal Ethics Committee of Fudan University Pudong Medical Center. We rinsed mouse bone marrow cavities using a complete medium containing 10% FBS and 1% P/S in RPMI 1640. The myelomonocytes were collected in sterile dishes. Then, the 2×10^6 myelomonocytes were cultured in a complete medium with 50 ng/mL active recombinant mouse GM-CSF protein and 50 ng/mL active recombinant mouse IL-4 protein. On the 3rd day of culture, the medium was replaced with half amount and supplemented with GM-CSF and IL-4. On the 5th day of culture, repeat the procedure. On the 7th day of culture, suspended and semi-suspended cells were collected, centrifuged at 1200 r/min for 5 min, resuspended in RPMI 1640 complete medium containing 50 ng/mL GM-CSF and 50 ng/mL IL-4, and reseeded into new sterile culture dishes. On the 9th day of culture, 5 μg/mL of mouse prostate cancer cell lysis antigen was added to the culture medium and cocultured with BMDC for 24 h. The suspended and semi-suspended cells were collected and detected by flow cytometry (BD FACSCaliburTM).

2.4 | Lysis and Extraction of Mouse Prostate Cancer Cell Antigens

The mouse prostate cancer RM-1 cells were collected and transferred to a frozen pipe. The frozen pipe was placed in liquid nitrogen for 15 min, then immediately placed in a 37°C water bath for 15 min, repeated four times. After lysis, the cells were centrifuged at 4°C at 12,000 r/min for 10 min. The supernatant was collected and filtered with a 0.22 μm filter to remove bacteria. The protein concentration was determined by the BCA method and stored at −80°C for later use.

2.5 | Flow Cytometry

BD FACSCalibur was used for flow cytometry of cell and tissue samples. FITC anti-mouse CD80 (104705), PE anti-mouse CD40 (157505), APC anti-mouse CD86 (105011), APC/FireTM 750 anti-mouse-I-A/I-E (107651) and Pacific BlueTM anti-mouse CD11c (117321) were used for the identification of mature BMDC. Antibodies to CD3, CD4, CD8, CD25, CD45, and Foxp3 were used to label T cells in the spleens of mice from different groups. All operations were performed in strict accordance with instructions.

2.6 | ELISA

Blood levels of IFN-γ and IL-2 in different groups of mice were determined by ELISA. The ELISA was performed using Abcam's Mouse IFN-γ ELISA kit (ab282874) and Mouse IL-2 ELISA Kit (ab100706). Absorbance was determined by enzyme-linked immunoassay (Tecan). All operations are carried out in strict accordance with the instructions.

2.7 | Animal Experiments

All animal experiments were carried out in a specific pathogen-free (SPF) animal laboratory. Male 6-week-old C57BL/6 mice were purchased from Shanghai Laboratory Animal Research Center (Shanghai, China). All mice were kept in an SPF animal laboratory and given clean water and food daily. ① Establishment of RM-1 mouse model of prostate cancer in situ. Mouse prostate cancer cell RM-1 was prepared with PBS into a cell suspension containing 1×10^8 cells per milliliter. The mice were intraperitoneally injected with 2% (w/v) pentobarbital (40 mg/kg) for anesthesia. A median abdominal incision of 1.5 cm was made to expose the prostate and bladder. With the help of an animal surgical microscope, the dorsal lobe of the prostate was exposed, and RM-l cell suspensions of 5 μL each were injected under the capsule of the left and right dorsal lobe of the prostate with a 25 μL microsyringe. The total number of cells was 1×10^6. The capsule at the injection site bulged up and formed a raised vesicle as the satisfactory standard. The prostate and bladder were restored to normal anatomical positions, and the incisions were closed with 3-0 silk-line discontinuity and double-layer suture. ② Mature BMDC induced by RM-1 cells antigen were transfused back into model mice after surgical resection of the primary tumor. Ultrasonography confirmed the successful establishment of an in situ tumor model of mouse prostate cancer cell RM-1. They were randomly divided into two groups with six mice in each group. The control group had the tumor surgically removed, leaving about one-fifth of the tumor at the surgical margin as a residual tumor. The experimental group underwent surgical resection of the tumor, leaving about one-fifth of the tumor as a residual tumor at the surgical margin, and injecting mature BMDC induced by RM-1 antigen on Days 3 and 7 after surgical treatment. The therapeutic effect of the two groups was evaluated by ultrasonography. ③ Mature BMDC induced by RM-1 cell antigen was transfused into normal mice, and RM-1 cells were inoculated subcutaneously. Mice in the control group were injected with mature BMDC induced by RM-1 antigen on Day 1 and Day 4, respectively, and 1×10^6 RM-1 cells were inoculated subcutaneously on Day 14. The experimental group of mice was injected with the same volume of PBS on Day 1 and Day 4, and 1×10^6 RM-1 cells were inoculated subcutaneously on Day 14.

2.8 | Ultrasound Imaging and Quantitative Analysis

Mice were monitored with ultrasound imaging after tumor implantation and treatment. To ensure the smooth progress of the experiment, all ultrasound scans were performed by the same experienced technician in the same equipment following standardized operating protocols. Mice were anesthetized with 2% pentobarbital and fixed on a heated platform. Ultrasound coupling gel was applied to the abdominal skin, and images of the prostate and surrounding area were obtained through the ventral body wall in the sagittal plane. During imaging, a body temperature was maintained between 36°C and 38°C.

Ultrasound imaging was performed using a high-resolution ultrasound system (SiliconWave 30, Kena Medical Technology Co., LTD). After focusing on the target region of the prostate, data acquisition began. Tumors were identified in the 3D ultrasound images, and continuous measurements were performed. The tumor size and volume measurements obtained were correlated with the macroscopic measurements. For the correlation analysis, tumor volume was calculated using the following formula: $V = \text{Length} \times \text{Width} \times \text{Height} \times \pi/6$ [16].

2.9 | Statistical Analysis

All experiments were repeated three times. In our study, GraphPad Prism (version 7, GraphPad Software) was used for statistical analysis. Comparisons between two groups were performed by Student's *t*-test for normally distributed variables or Mann–Whitney *U* test for nonnormally distributed variables. One-way ANOVA with Tukey's post hoc test was used to determine whether the differences among several groups were statistically significant. Values of $p < 0.05$ were considered to indicate statistically significant differences.

3 | Results

3.1 | RM-1 Cell Antigen Induces Mature Dendritic Cells

Mouse bone marrow mononuclear cells were isolated from the femur and tibia and induced to differentiate with the cytokines GM-CSF and IL-4. The antigen of mouse prostate cancer cell RM-1 was extracted by rapid liquid nitrogen freezing method and added to mature dendritic cells for coculture. Figure 1A shows the morphology of induced mature dendritic cells, which protrudes dendritic-like processes on the cell surface, have irregular morphology, are large and transparent, and the cells show aggregated growth. Our results showed that dendritic cells supplemented with RM-1 antigen had higher maturity and expressed more CD80 and CD86 antigens on the cell surface (Figure 1B,C).

3.2 | Induction of Mature Dendritic Cells With RM-1 Cell Antigen Significantly Inhibits the Growth of Orthotopic and Subcutaneous Prostate Cancer in Mice

We used ultrasound to evaluate the therapeutic effect of dendritic cell injection on prostate cancer in situ in mice.

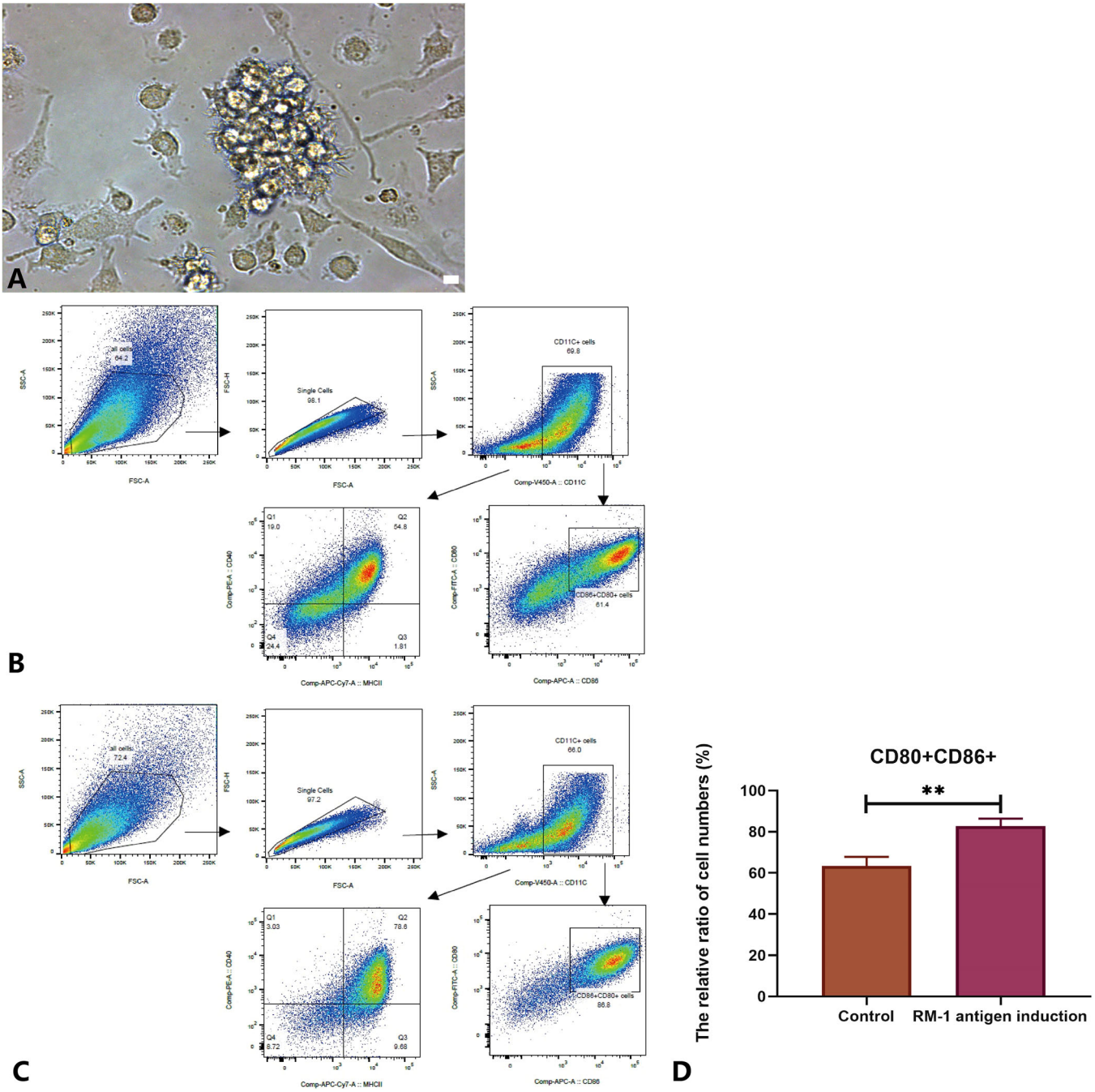
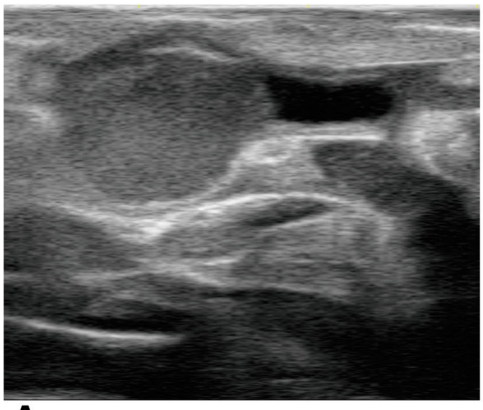


FIGURE 1 | (A) The morphology of induced mature dendritic cells, which protrudes dendritic-like processes on the cell surface, has irregular morphology, is large and transparent, and the cells show aggregated growth. (B) Dendritic cell surface antigen expression without RM-1 antigen induction. (C) Surface antigen expression of dendritic cells induced by RM-1 antigen. (D) Proportion of dendritic cells expressing both CD80 and CD86 surface antigens between the two groups. $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

The results showed that mature dendritic cells induced by RM-1 antigen exhibited a significant inhibitory effect on prostate cancer in mice (Figure 2A–C). We then investigated the effect of injection of mature dendritic cells on subcutaneous tumorigenesis of mouse RM-1 cells. The results showed that injection of mature dendritic cells induced by RM-1 antigen significantly inhibited subcutaneous tumor growth compared with the control group (Figure2D–F).

3.3 | After Injection of Dendritic Cells Induced by RM-1 Antigen, CD4 + T Cells and Treg Cells Have Increased Significantly

We used flow cytometric analysis to detect different types of immune T cells in the spleen after dendritic cell injection in mice prostate carcinoma in situ model. The results showed that the levels of CD4 + T cells and Treg cells were significantly elevated after injection of dendritic cells induced by RM-1



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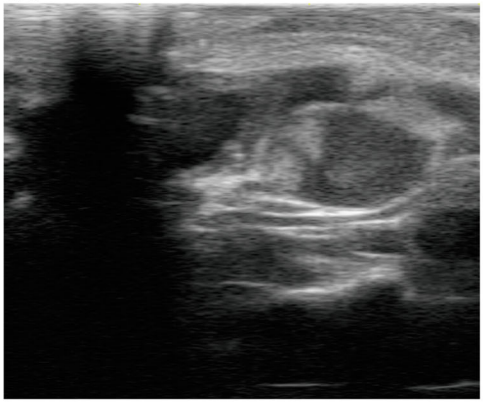

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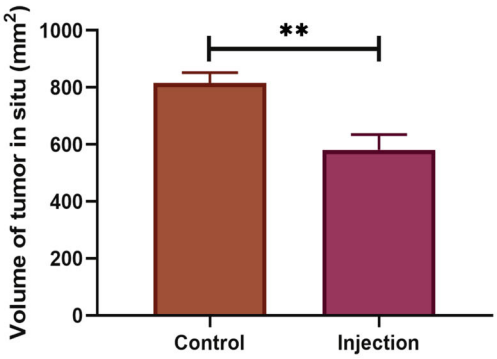

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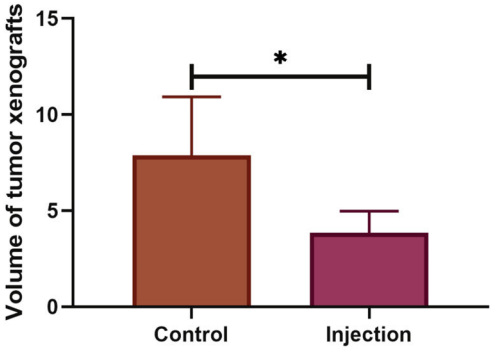

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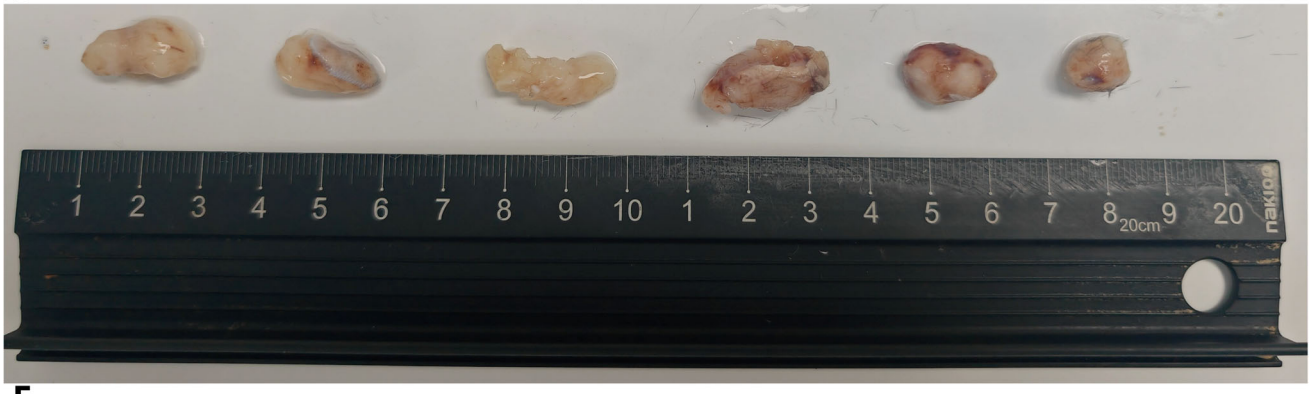

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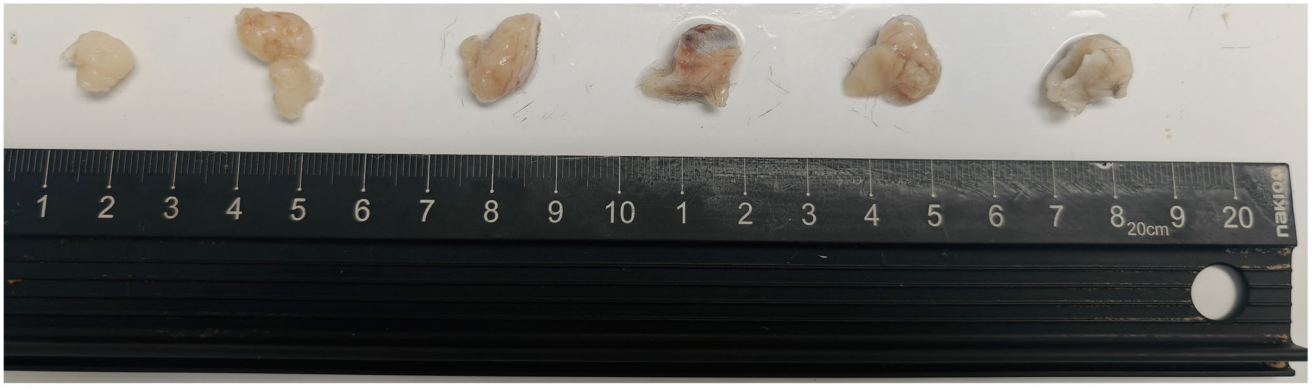

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FIGURE 2 | (A) Ultrasound images of prostate cancer in situ in control mice. (B) Ultrasound images of prostate cancer in situ in mice injected with mature dendritic cells induced by RM-1 cell antigen. (C) Volume analysis of prostate cancer in situ between the two groups. (D) Volume analysis of subcutaneous tumors of RM-1 cells between the two groups. (E) Image of RM-1 cell subcutaneous tumors in control group. (F) Image of RM-1 cell subcutaneous tumors in group receiving dendritic cell injection. $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

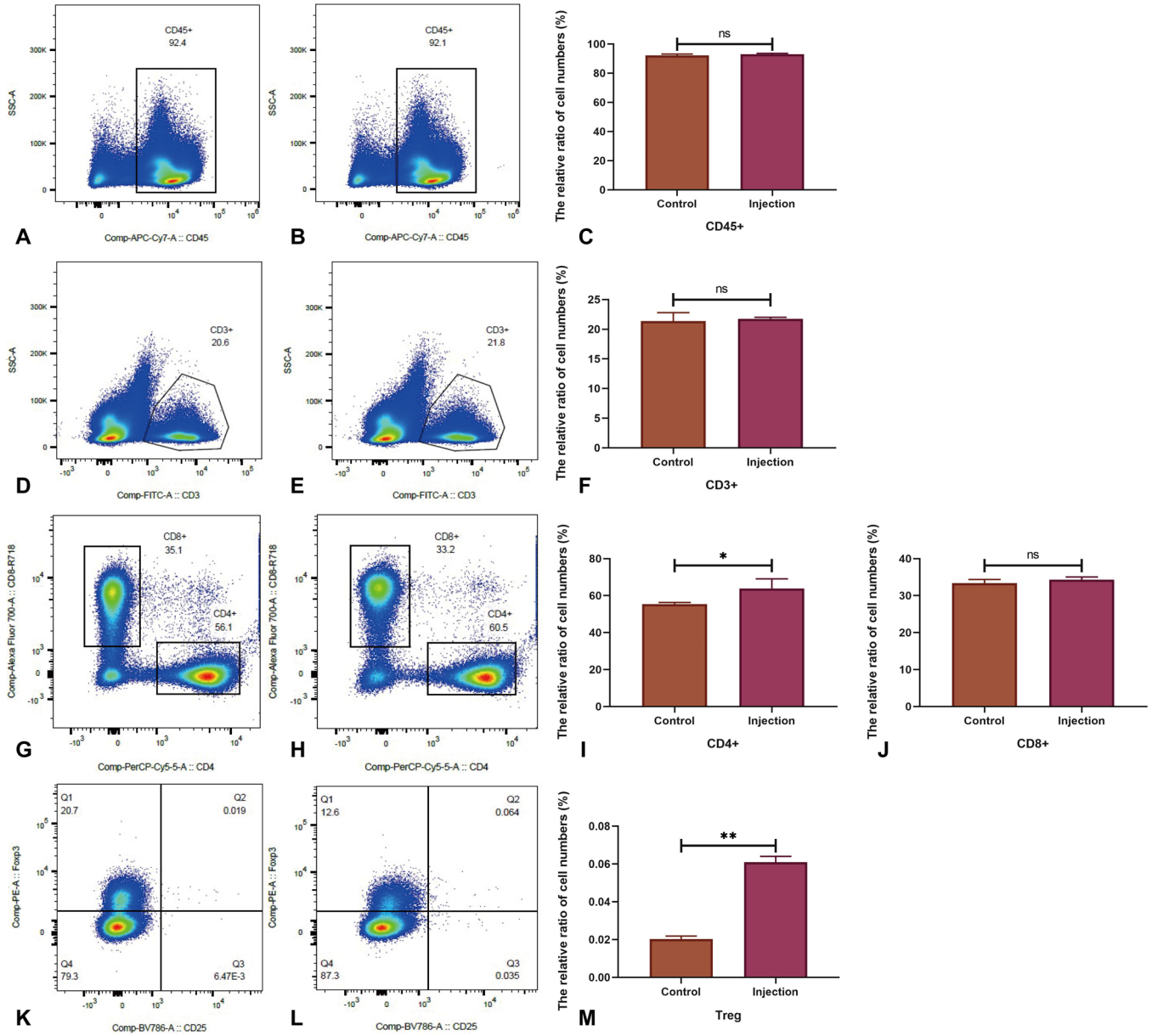

FIGURE 3 | (A, D, G, K) Results of flow cytometric analysis of CD45 + T cells, CD3 + T cells, CD8 + T cells, CD4 + T cells, and Treg cells in the spleens of control group mice. (B, E, H, L) Results of flow cytometric analysis of CD45 + T cells, CD3 + T cells, CD8 + T cells, CD4 + T cells, and Treg cells in spleens of mice in the dendritic cell injection group. (C, F, I, J, M) Statistical analysis of flow cytometry results of different types of T cells in the spleens of mice in control and dendritic cell injection group. *$p < 0.05$, ns: no significance. [Color figure can be viewed at wileyonlinelibrary.com]

antigen (Figure 3G–I,K–M). However, there were no significant changes in the levels of CD45 + T cells, CD3 + T cells, and CD8 + T cells (Figure 3A–F,G,H,J).

3.4 | Injection of Mature Dendritic Cells Induced by RM-1 Cell Antigen Significantly Increased the Levels of IL-2 and IFN-γ in the Peripheral Blood of Mice

We measured IL-2 and IFN-γ levels in the peripheral blood of mice after injection of mature dendritic cells by ELISA. Injection of mature dendritic cells induced by RM-1 cell antigen resulted in higher levels of IL-2 and IFN-γ in the peripheral blood of mice compared with the control group. Consistent results were obtained in the mouse prostate cancer in situ and subcutaneous tumor models (Figure 4A–D).

4 | Discussion

Prostate cancer has become a major problem that endangers the health of men in the world, threatening the safety of men's life, and the incidence is rising year by year [17]. At present, the treatment of prostate cancer mainly includes radical prostatectomy (RP), chemotherapy, radiotherapy, and endocrine therapy. RP has a good effect on early prostate tumors, but the biochemical recurrence rate of 5 years after the implementation of RP is not satisfactory [18]. Dendritic cells, as the most powerful cells in the immune system that perform antigen-presenting

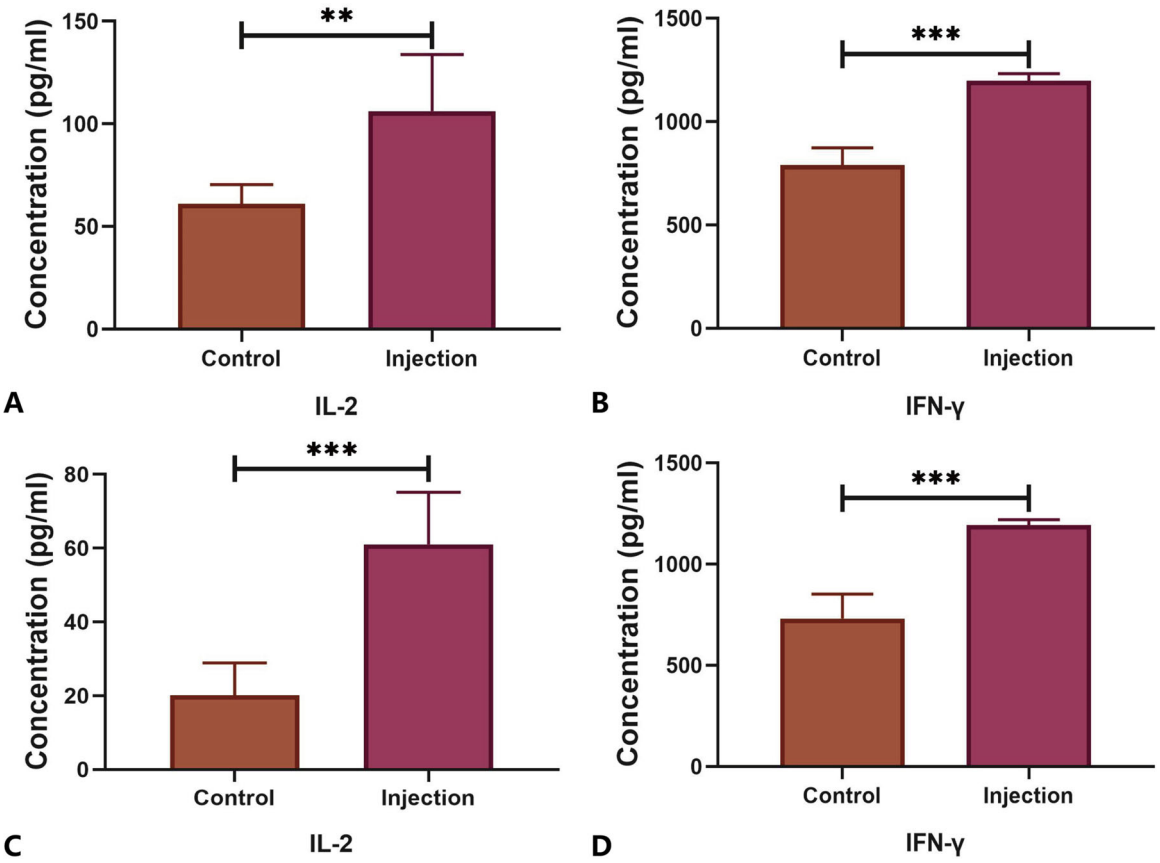

FIGURE 4 | (A, B) ELISA results of IL-2 and IFN-γ in the peripheral blood of the prostate cancer in situ model mice. (C, D) ELISA results for IL-2 and IFN-γ in peripheral blood from RM-1 cell subcutaneous tumor model mice. $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

functions, have attracted great attention in the field of tumor therapy [19]. Dendritic cells play an active anticancer role in the tumor microenvironment, processing tumor antigens and presenting them to T cells, linking intrinsic and adaptive immunity [20]. The antigen-presenting role of dendritic cells is dependent on their differentiation and maturation, and mature differentiated dendritic cells highly express CD80 and CD86 antigens on their surface [21], in our study, we extracted antigens from mouse prostate cancer cells RM-1 and cocultured them with immature dendritic cells. The results showed that the expression of CD80 and CD86 antigens on the surface of dendritic cells was significantly increased after 24 h of stimulation with RM-1 antigens, suggesting that it is feasible to stimulate the maturation of dendritic cells in vitro by lysing the tumor cells to extract antigens.

In our study, injection of mature dendritic cells induced by RM-1 antigen after RP in mice significantly retarded the growth of stump tumors; similarly, consistent results were obtained in mice subcutaneous tumor model. Studies have been done to discuss the role of dendritic cells as a tumor vaccine [22], and we have obtained consistent results. In addition to acting as a tumor vaccine, our study proposes a new therapeutic modality of applying dendritic cells as an adjunct to surgical treatment. Dendritic cells can present antigens to different types of T cells and stimulate their activation to exert T cell responses [23]. In our study, the proportion of CD4 + T cells increased significantly after receiving dendritic cell injections, whereas there was no significant change in CD8 + T cells, which may be due to the fact that in the tumor microenvironment, helper T cells are more inclined to interact with dendritic cells than CD8 + T cells [24], and thus we monitored the increase in the proportion of CD4 + T cells first. Despite the fact that CD8 + T cells act as the main effector cells mediating tumor immunity [25], we did not monitor significant changes, probably because of the time of sampling. In our study, Treg cells rose significantly after receiving dendritic cell injections, which may be related to the rise in CD4 + T cells, which, as part of the CD4+ cells, are responsible for regulating the cessation of the immune system and preventing the body from becoming over-immunized [26]. CD3 + T cells and CD45 + T cells represent the level of overall T cells, our results suggest that antigen-induced maturation of dendrites in vitro has no significant effect on overall T cells [27]. Interleukins are an important class of cytokines that play important roles in inflammation, metabolism, immunity, and tumorigenesis and development. IL-2, mainly produced by CD4+ and CD8 + T cells, has the effect of activating T cells, promoting cytokine production, stimulating the proliferation and activation of NK cells, inducing the production of LAK cells, promoting the proliferation of activated B cells and the production of antibodies, and activating monocyte-macrophages, etc. [28]. IFN-γ is a cytokine produced by T cells and NK cells with broad-spectrum antiviral, antitumor, and immunomodulatory effects [29]. In our study, the expression levels of IL-2 and IFN-γ were significantly elevated after receiving injections of mature dendritic cells induced by RM-1

antigen, suggesting that T cells activated by dendritic cells are functionally active and secrete elevated levels of relevant immunomodulatory factors, which in turn can further stimulate the activation of effector T cells.

Currently, there have been clinical studies on autologous DC-based active immunotherapy for prostate cancer, which can improve overall survival in patients. Despite initial success, the clinical application of DC remains limited. Currently, the majority of DC therapy is mainly used for asymptomatic or mild mCRPC, as seen in studies by Podrazil M, Adam S Kibel, and Vogelzang NJ [30–32]. In addition, Thomas-Kaskel AK reported that DC therapy was performed in advanced prostate cancer patients, with only one case of complete remission [33]. There is very limited research involving DC therapy after RP for prostate cancer. Furthermore, antigen-induced mature DC was used in this study. A meta-analysis of 10 clinical trials of DC vaccines in prostate cancer confirmed that immature DC reduced the ability to stimulate T cells, resulting in lower clinical benefit [34]. Antigen-loaded DC released by the host's own prostate tumor tissue can serve as an autologous vaccine, overcoming the limitations of single antigen-loaded DC. As the immune function of the body does not significantly decline early after prostate cancer surgery, DC therapy can effectively enhance the host's specific antitumor immune response. Therefore, the combination therapy proposed in this study has certain innovation and potential in terms of mechanisms, therapeutic efficacy, and preclinical research of prostate cancer.

5 | Conclusions

Our study presents a new potential way of utilizing dendritic cells to treat prostate cancer as an adjuvant therapy to surgical treatment and was successfully validated in mice model. Surgical treatment combined with immunotherapy is a new strategy for treating prostate cancer in the future compared to conventional treatment.

Acknowledgments

The authors have nothing to report.

Ethics Statement

All animal experiments in this study were approved by the Animal Ethics Committee of Fudan University Pudong Hospital (Grant. No: 2019-QKWXM-01) and were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and materials in this study are available for scientific research.

References

1. T. Li, Y. Li, T. Liu, et al., "Mitochondrial PAK6 Inhibits Prostate Cancer Cell Apoptosis via the PAK6-SIRT4-ANT2 Complex," *Theranostics* 10 (2020): 2571–2586.

2. X. Xu, Z. Zhu, Y. Xu, S. Tian, Y. Jiang, and H. Zhao, "Effects of Zinc Finger Protein 403 on the Proliferation, Migration and Invasion Abilities of Prostate Cancer Cells," *Oncology Reports* 44 (2020): 2455–2464.

3. S.-R. Lin, H.-L. Yeh, and Y.-N. Liu, "Interplay of Epidermal Growth Factor Receptor and Signal Transducer and Activator of Transcription 3 in Prostate Cancer: Beyond Androgen Receptor Transactivation," *Cancers* 13 (2021): 3452.

4. Y. Ye, S.-L. Li, and J.-J. Wang, "miR-100-5p Downregulates mTOR to Suppress the Proliferation, Migration, and Invasion of Prostate Cancer Cells," *Frontiers in Oncology* 10 (2020): 578948.

5. C. Tonry, S. Finn, J. Armstrong, and S. R. Pennington, "Clinical Proteomics for Prostate Cancer: Understanding Prostate Cancer Pathology and Protein Biomarkers for Improved Disease Management," *Clinical Proteomics* 17 (2020): 41.

6. C. Suárez, R. Morales-Barrera, V. Ramos, et al., "Role of Immunotherapy in Castration-Resistant Prostate Cancer (CRPC)," *BJU International* 113 (2014): 367–375.

7. A. J. Costello, "Considering the Role of Radical Prostatectomy in 21st Century Prostate Cancer Care," *Nature Reviews Urology* 17 (2020): 177–188.

8. A. Sımsır, C. Cal, R. Mammadov, I. Cureklıbatır, B. Semercı, and G. Gunaydın, "Biochemical Recurrence After Radical Prostatectomy: Is the Disease or the Surgeon to Blame?," *International Braz J Urol: Official Journal of the Brazilian Society of Urology* 37 (2011): 328–334.

9. N. D. Shore, L. Karsh, L. G. Gomella, T. E. Keane, R. S. Concepcion, and E. D. Crawford, "Avoiding Obsolescence in Advanced Prostate Cancer Management: A Guide for Urologists," *BJU International* 115 (2015): 188–197.

10. Y. Jiang, W. Wen, F. Yang, D. Han, W. Zhang, and W. Qin, "Prospect of Prostate Cancer Treatment: Armed CAR-T or Combination Therapy," *Cancers* 14 (2022): 967.

11. Z. X. Xiao, X. Hu, X. Zhang, et al., "High Salt Diet Accelerates the Progression of Murine Lupus Through Dendritic Cells via the p38 MAPK and STAT1 Signaling Pathways," *Signal Transduction and Targeted Therapy* 5 (2020): 34.

12. M. López González, D. Oosterhoff, J. J. Lindenberg, et al., "Constitutively Active GSK3β as a Means to Bolster Dendritic Cell Functionality in the Face of Tumour-Mediated Immune Suppression," *Oncoimmunology* 8 (2019): e1631119.

13. Y. Qian, Q. Liu, P. Li, et al., "Highly Tumor-Specific and Long-Acting Iodine-131 Microbeads for Enhanced Treatment of Hepatocellular Carcinoma With Low-Dose Radio-Chemoembolization," *ACS Nano* 15 (2021): 2933–2946.

14. M. A. Cheever and C. S. Higano, "PROVENGE (Sipuleucel-T) in Prostate Cancer: The First FDA-Approved Therapeutic Cancer Vaccine," *Clinical Cancer Research* 17 (2011): 3520–3526.

15. E. S. Antonarakis, E. J. Small, D. P. Petrylak, et al., "Antigen-Specific CD8 Lytic Phenotype Induced by Sipuleucel-T in Hormone-Sensitive or Castration-Resistant Prostate Cancer and Association With Overall Survival," *Clinical Cancer Research* 24 (2018): 4662–4671.

16. M. Saar, C. Körbel, V. Jung, et al., "Experimental Orthotopic Prostate Tumor In Nude Mice: Techniques for Local Cell Inoculation and Three-Dimensional Ultrasound Monitoring," *Urologic Oncology: Seminars and Original Investigations* 30 (2012): 330–338.

17. H. Jadvar, "Molecular Imaging of Prostate Cancer With 18F-fluorodeoxyglucose PET," *Nature Reviews Urology* 6 (2009): 317–323.

18. C. Siech, M. Wenzel, N. Grosshans, et al., "The Association Between Lymphovascular or Perineural Invasion in Radical Prostatectomy Specimen and Biochemical Recurrence," *Cancers* 16 (2024): 3648.

19. X. Song, Y. Jiang, W. Zhang, et al., "Transcutaneous Tumor Vaccination Combined With Anti-Programmed Death-1 Monoclonal Antibody Treatment Produces a Synergistic Antitumor Effect," *Acta Biomaterialia* 140 (2022): 247–260.

20. U. T. T. Than, H. T. Le, D. H. Hoang, et al., "Induction of Antitumor Immunity by Exosomes Isolated From Cryopreserved Cord Blood Monocyte-Derived Dendritic Cells," *International Journal of Molecular Sciences* 21 (2020): 1834.

21. B. Bodey, S. E. Siegel, and H. E. Kaiser, "Antigen Presentation by Dendritic Cells and Their Significance in Antineoplastic Immunotherapy," *In Vivo* 18 (2004): 81–100.

22. C. R. Perez and M. De Palma, "Engineering Dendritic Cell Vaccines to Improve Cancer Immunotherapy," *Nature Communications* 10 (2019): 5408.

23. A. Lanzavecchia and F. Sallusto, "The Instructive Role of Dendritic Cells on T Cell Responses: Lineages, Plasticity and Kinetics," *Current Opinion in Immunology* 13 (2001): 291–298.

24. M. Cohen, A. Giladi, O. Barboy, et al., "The Interaction of CD4+ Helper T Cells With Dendritic Cells Shapes the Tumor Microenvironment and Immune Checkpoint Blockade Response," *Nature Cancer* 3 (2022): 303–317.

25. F. Mami-Chouaib, C. Blanc, S. Corgnac, et al., "Resident Memory T Cells, Critical Components in Tumor Immunology," *Journal for Immunotherapy of Cancer* 6 (2018): 87.

26. M. A. A. Claassen, R. J. de Knegt, H. L. A. Janssen, and A. Boonstra, "Retention of CD4+ CD25+ FoxP3+ Regulatory T Cells in the Liver After Therapy-Induced Hepatitis C Virus Eradication in Humans," *Journal of Virology* 85 (2011): 5323–5330.

27. H. Hou, Y. Luo, G. Tang, et al., "Dynamic Changes In Peripheral Blood Lymphocyte Subset Counts and Functions in Patients With Diffuse Large B Cell Lymphoma During Chemotherapy," *Cancer Cell International* 21 (2021): 282.

28. H. Shi, W. Wang, J. Yin, et al., "The Inhibition of IL-2/IL-2R Gives Rise to CD8+ T Cell and Lymphocyte Decrease Through JAK1-STAT5 in Critical Patients With COVID-19 Pneumonia," *Cell Death & Disease* 11 (2020): 429.

29. L. Zheng, Q. Liu, R. Li, et al., "Targeting MDK Abrogates IFN-γ-Elicited Metastasis in Cancers of Various Origins," *Frontiers in Oncology* 12 (2022): 885656.

30. M. Podrazil, R. Horvath, E. Becht, et al., "Phase I/II Clinical Trial of Dendritic-Cell Based Immunotherapy (DCVAC/PCA) Combined With Chemotherapy In Patients With Metastatic, Castration-Resistant Prostate Cancer," *Oncotarget* 6 (2015): 18192–18205.

31. A. S. Kibel, B. A. Inman, R. K. Pachynski, T. Vu, N. A. Sheikh, and D. P. Petrylak, "Videos of Sipuleucel-T Programmed T Cells Lysing Cells That Express Prostate Cancer Target Antigens," *JNCI: Journal of the National Cancer Institute* 114 (2022): 310–313.

32. N. J. Vogelzang, T. M. Beer, W. Gerritsen, et al., "Efficacy and Safety of Autologous Dendritic Cell-Based Immunotherapy, Docetaxel, and Prednisone vs Placebo in Patients With Metastatic Castration-Resistant Prostate Cancer: The Viable Phase 3 Randomized Clinical Trial," *JAMA Oncology* 8 (2022): 546.

33. A.-K. Thomas-Kaskel, C. F. Waller, W. Schultze-Seemann, and H. Veelken, "Immunotherapy With Dendritic Cells for Prostate Cancer," *International Journal of Cancer* 121 (2007): 467–473.

34. A. Draube, N. Klein-González, S. Mattheus, et al., "Dendritic Cell Based Tumor Vaccination in Prostate and Renal Cell Cancer: A Systematic Review and Meta-Analysis," *PLoS One* 6 (2011): e18801.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.