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Irreversible electroporation synergizes with oncolytic virus enhances the infiltration of cytotoxic T lymphocytes in the tumor immune microenvironment: a leap from focal therapy to immunotherapy for prostate cancer

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ABSTRACT

Irreversible electroporation (IRE), a non-thermal focal therapy, employs high-frequency electrical pulses to create tumor cell membrane nanopores, inducing immunogenic cell death. By triggering damage-associated molecular patterns, IRE activates dendritic cells (DCs) to prime systemic cytotoxic T lymphocyte (CTL) responses. However, IRE-activated CTLs predominantly accumulate in perivascular regions, limiting their infiltration into the tumor parenchyma. Oncolytic viruses (OVs) complement IRE by converting the "primed" but spatially restricted CTL response into a robust, intratumoral immune attack. OVs secrete pro-inflammatory chemokines to chemoattract CTLs into the tumor core, while the non-thermal nature of IRE preserves antigen stability and vascular integrity, collectively sustaining DC activation and CTL infiltration. Moreover, intraoperative ultrasound-guided IRE not only establishes physical channels for precise OVs delivery but also enhances tumor cell susceptibility to OVs by disrupting membrane integrity. By bridging focal ablation with adaptive immunity, the synergistic effect—IRE for systemic CTL priming and OVs for localized immune amplification—transforms residual tumors into immunogenic niches, counteracting post-IRE recurrence through durable CTL-mediated clearance. The strategy leverages IRE's functional preservation and OVs' targeted lytic activity to synergistically enhance therapeutic efficacy while minimizing invasiveness.

Prostate cancer (PCa), a prevalent malignant neoplasm within the male urogenital system, benefits from the widespread implementation of prostate-specific antigen screening and the enhanced public health awareness. Consequently, a substantial proportion of patients are not diagnosed with localized or low/intermediate risk PCa at their first visit. For low-risk patients, active surveillance or the watchful waiting approach has become the standard of care. For intermediate-risk patients, radical prostatectomy is the treatment of choice, effectively controlling tumor progression. However, despite the surgery's significant efficacy in controlling the tumor, it may have an inevitable negative impact on the patient's urinary and erectile functions, severely diminishing their quality of life. Consequently, the pursuit of novel treatment that can mitigate the risk of complications and elevate the therapeutic efficacy remains an ongoing area of research and clinical investigation.

Irreversible electroporation (IRE) has emerged as a promising treatment modality for PCa, offering a safer alternative to traditional radical procedures. By delivering high-frequency, high-potential electrical pulses, IRE creates permanent membrane perforation, leading to tumor cell lysis. This method stands out among other focal or physical treatment modalities for its non-thermal advantages, precluding thermal or cryogenic injury to neighboring tissues and organs. Moreover, the heat sink effect—the thermal energy dissipation in the target area—rendered negligible. IRE also offers superior protection for blood vessels and nerves, as the higher voltage thresholds required for IRE in vascular endothelial and nerve cells mean that standard treatment parameters do not cause irreversible damage to these cells. The

feature is particularly beneficial considering the proximity of vital structures like the bladder, rectum, and nerves in the prostate region.

In a follow-up study enrolling 123 patients treated with IRE therapy, 18 patients (14.6%) observed a clinically significant recurrence of PCa [International Society of Urological Pathology (ISUP) grade ≥2] within 12 months of treatment. Most patients (98.8%) maintained pad-free status, and 76% reported no change in erectile function.[1] Furthermore, a multicenter European study included 106 patients with localized PCa, of which 51 underwent focal ablation and 55 underwent extended ablation. A prostate biopsy conducted 30 months after treatment showed a recurrence rate of 18.8% in the focal ablation group and 13.2% in the extended ablation group. Although there was no statistically significant difference in recurrence rates between the two groups, it is worth noting that no Clavien-Dindo grade III–IV adverse events were reported postoperatively in either group.[2] The factors contributing to the unsatisfactory recurrence rate after electroporation include the intrinsic heterogeneity of voltage tolerance in prostate tumors and the potential for residual tumor due to the diffraction of electric fields within the target area. When the applied electrical stimulation is inadequate to induce tumor cell death, these residual tumor cells exhibit a transient increase in membrane permeability, facilitating the uptake of macromolecules and virus particles from the extracellular milieu. This process is referred to as electroporation-mediated transfection. Leveraging this unique attribute of electroporation, we propose the following hypothesis: IRE synergizes with oncolytic viruses (OVs) to enhance immunotherapy for PCa (figure 1).

Effective immunotherapy relies on the immune response within the tumor microenvironment, primarily involving the following aspects: (1) a high density of cytotoxic T lymphocytes (CTLs) ensures a robust tumor immune killing effect; (2) the infiltrating CTLs effectively recognize tumor-specific antigens; (3) the suppressive effects on tumor immunotherapy within the tumor microenvironment (TME) are alleviated.[3] The limited mutational burden in PCa indicates a reduced potential for immunotherapy,[4] particularly in targeting neoantigens. This lower mutation rate implies a reduced likelihood of generating unique, immunogenic tumor antigens, which are crucial for eliciting effective antitumor immune responses. Consequently, immunotherapeutic strategies may need to focus on enhancing tumor immunogenicity to overcome the challenges posed by the low mutational burden in PCa. Compared with normal healthy cells, tumor cells often exhibit significant antiviral functional defects,

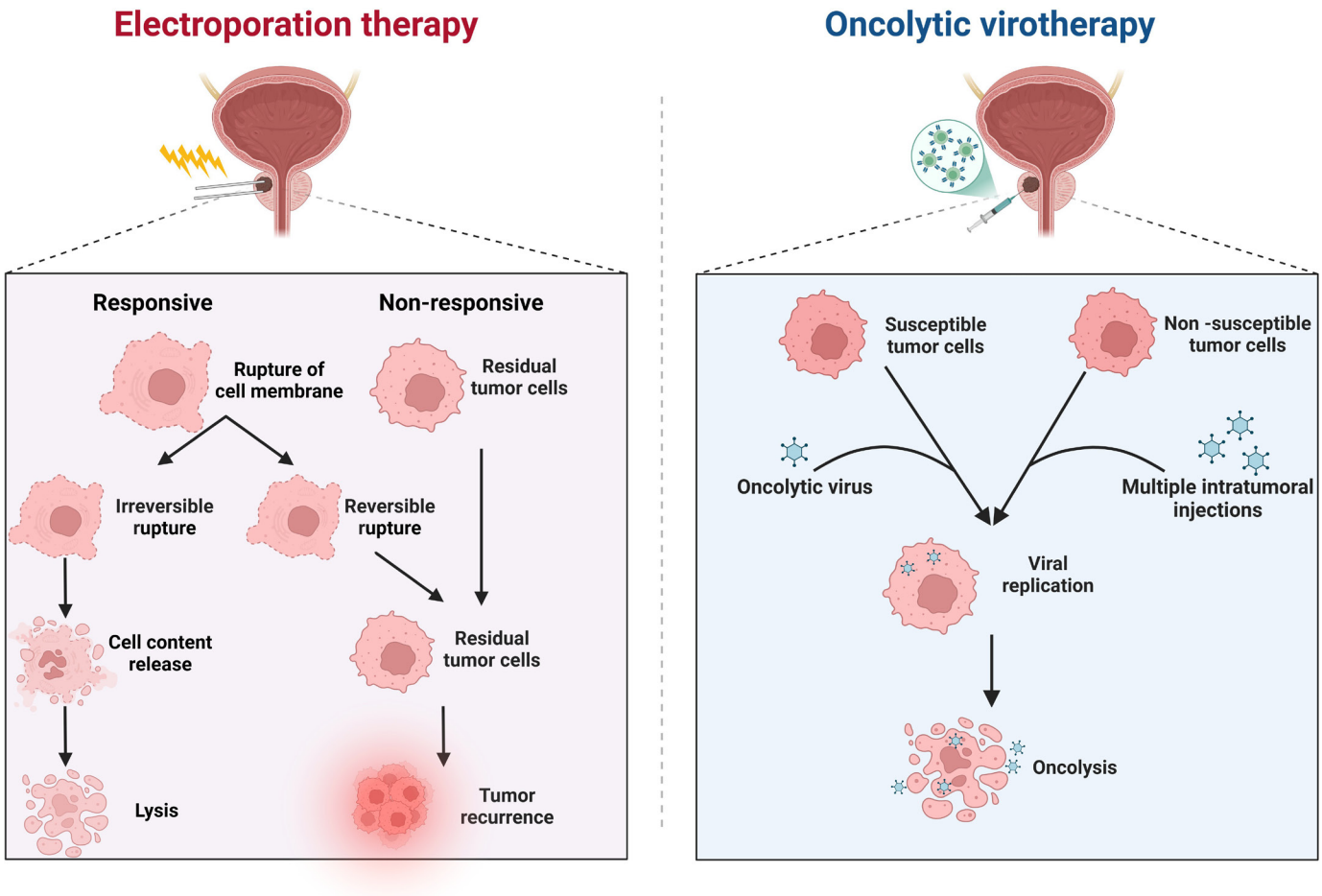

Figure 1 The limitations and challenges of electroporation therapy and oncolytic virotherapy.

providing favorable conditions for the smooth replication and packaging of OVs. OVs possess the capability to directly eliminate tumor cells, subsequently leading to the release of tumor-associated antigens. The release of these antigens is crucial for subsequent immune responses, as they attract and activate antigen-presenting cells, particularly BATF3^{+} dendritic cell (DC) cells. These cells phagocytose the antigens and migrate them to regional lymph nodes, triggering a specific adaptive T-cell response against the tumor. Furthermore, the interferon-γ induced by OVs plays a pivotal role in antitumor immunity. It not only upregulates the expression of major histocompatibility complex I molecules, enhancing the immunogenicity of tumor cells, but also promotes the recruitment of tumor-specific infiltrating CD8^{+} T cells. These CTLs possess powerful killing abilities, capable of recognizing and eliminating tumor cells, including those located at distal sites. Therefore, OVs not only directly kill tumor cells but also enhance antitumor immune effects through the activation of the immune system, achieving the dual role of neoantigen presentation and tumor-specific T-lymphocyte infiltration. The efficacy of OVs therapy currently hinges on multiple intratumoral injections. However, this therapeutic approach raises concerns about the feasibility and compliance of such an invasive approach, given the anatomical depth of the prostate within the perineal area and the necessity for repeated needle puncture injection. Additionally, the off-target effect can lead to a decrease in viral transduction efficiency, thereby affecting the therapeutic outcome. Theoretically, when OVs are combined with electroporation therapy, the viruses can be directly delivered to the target area of PCa during the electroporation process, eliminating the need for additional needle puncture injection. Simultaneously, electroporation enhances the transfection efficiency of the OVs, thereby amplifying the therapeutic effect (figure 2).

The synergistic interplay between IRE and OVs in PCa operates through three interlinked immunological axes: (1) antigen priming and release, (2) chemokine-driven lymphocyte recruitment, and (3) immunosuppressive TME reprogramming. First, IRE induces immunogenic cell death via non-thermal membrane permeabilization, releasing tumor-associated antigens (TAAs) and damage-associated molecular patterns. These signals activate DCs, initiating systemic CTLs responses. However, stromal barriers (eg, dense extracellular matrix) restrict CTLs infiltration, leaving residual tumors prone to recurrence. Here, OVs act as force multipliers: by lysing IRE-damaged cells, they amplify antigen release and deliver exogenous genes (eg, Granulocyte-macrophage colony-stimulating

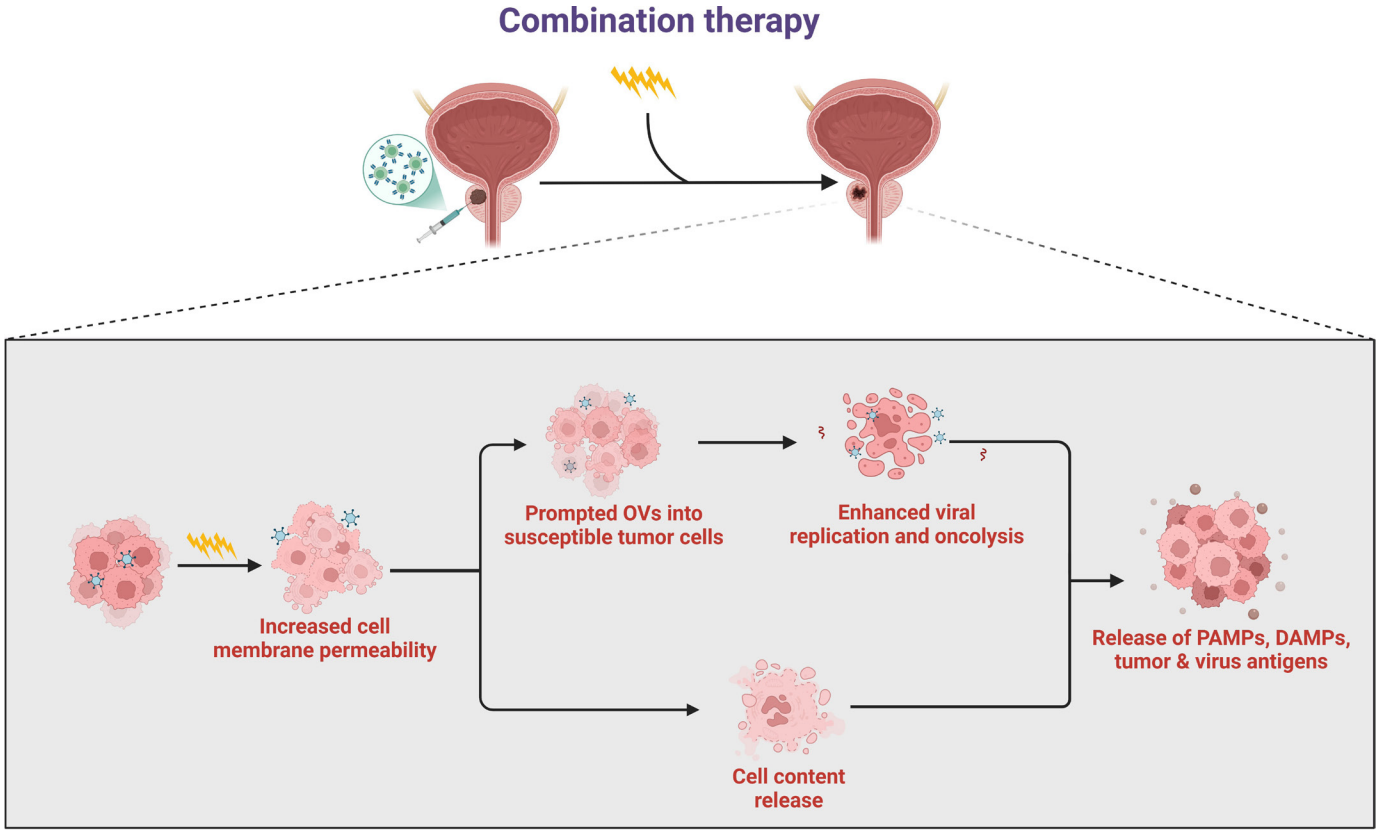

Figure 2 Electroporation combined with OVs: a novel immunotherapy strategy for PCa. Electroporation-mediated transfection facilitates the delivery of OVs into the tumor cell. Subsequently, OVs lead to tumor cell lysis and the release of tumor-associated antigens, which is further enhanced by the vascular and antigen preservation effect of irreversible electroporation. The synergistic interaction leads to a robust antitumor immune response, characterized by an increased density of cytotoxic T lymphocytes within the tumor microenvironment, effective recognition of tumor-specific antigens, and the alleviation of immunosuppressive effects. DAMPs, damage-associated molecular patterns; OVs, oncolytic viruses; PCa, prostate cancer.

factor(GM-CSF)) to transform infected cells into in situ vaccines.[5] Critically, IRE's non-thermal property preserves antigen integrity and vascular networks, ensuring DCs remain activated and CTLs can traffic to tumor targets—a limitation of thermal ablation modalities.[6] Second, while IRE primes CTLs systemically, their perivascular sequestration necessitates localized chemotactic guidance. OVs address this by secreting CXCL9/CXCL10 (ligands for CXCR3 on CTLs) creating chemokine gradients that direct CTLs into the tumor parenchyma. Ultrasound-guided IRE further optimizes this process by: disrupting physical barriers: electroporation-generated nanopores enhance OV penetration and replication in residual tumor; preserving chemokine stability: non-thermal ablation prevents ECM degradation, maintaining chemokine gradients critical for CTL navigation. Simultaneously, the protective effect of blood vessels of electroporation creates a conducive environment for the infiltration of tumor lymphocytes, further facilitating the immune response against the PCa. Lastly, the suppressive effects on tumor immunotherapy within the TME can be alleviated by this combination treatment. The tumor immune microenvironment is characterized by the presence of immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages, and regulatory T cells. Katayama *et al*[7] demonstrated that in melanoma or lymphoma mouse models, oncolytic reovirus can be engulfed by MDSCs. Subsequently, the double-stranded DNA of the virus is recognized by toll-like receptor 3 (TLR3), ultimately resulting in the alleviation of the immunosuppressive function of MDSCs, and this effect does not depend on viral replication or host cell lysis. Electroporation has been demonstrated to alter the hypoxic and acidic environment in the TME,[8] thereby increasing extracellular pH, which in turn facilitates the binding affinity of dsDNA and TLR3.[9] Concurrently, hypoxia alleviation improves CTL metabolic fitness, enabling sustained tumoricidal activity. Importantly, this combination avoids the "immune deserts" typical of monotherapy, where residual tumors evade detection. While the exact balance between anti-viral and tumor-specific immune responses in the IRE-OVs combination therapy remains an area of ongoing research, our hypotheses, along with existing evidence,[10] suggest that OVs can effectively induce tumor-specific immune responses leading to the activation of CTLs and systemic protection against cancer.

In summary, by bridging IRE's precision ablation with OVs' immunostimulatory potency, this strategy transcends the limitations of focal therapy, converting transient tumor debulking into durable systemic immunity. As mechanistic insights evolve, tailored combination regimens promise to redefine PCa management—shifting the paradigm from localized treatment to immune-centric cure.

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REFERENCES

1 Blazevski A, Scheltema MJ, Yuen B, *et al*. Oncological and Quality-of-life Outcomes Following Focal Irreversible Electroporation as Primary Treatment for Localised Prostate Cancer: A Biopsy-monitored Prospective Cohort. *Eur Urol Oncol* 2020;3:283–90.
2 Zhang K, Teoh J, Laguna P, *et al*. Effect of Focal vs Extended Irreversible Electroporation for the Ablation of Localized Low- or Intermediate-Risk Prostate Cancer on Early Oncological Control: A Randomized Clinical Trial. *JAMA Surg* 2023;158:343–9.
3 Twumasi-Boateng K, Pettigrew JL, Kwok YYE, *et al*. Oncolytic viruses as engineering platforms for combination immunotherapy. *Nat Rev Cancer* 2018;18:419–32.
4 Wang L, Pan S, Zhu B, *et al*. Comprehensive analysis of tumour mutational burden and its clinical significance in prostate cancer. *BMC Urol* 2021;21:29.
5 Zuo S, Wei M, Xu T, *et al*. An engineered oncolytic vaccinia virus encoding a single-chain variable fragment against TIGIT induces effective antitumor immunity and synergizes with PD-1 or LAG-3 blockade. *J Immunother Cancer* 2021;9:e002843.
6 van Vloten JP, Workenhe ST, Wootton SK, *et al*. Critical Interactions between Immunogenic Cancer Cell Death, Oncolytic Viruses, and the Immune System Define the Rational Design of Combination Immunotherapies. *J Immunol* 2018;200:450–8.
7 Katayama Y, Tachibana M, Kurisu N, *et al*. Oncolytic Reovirus Inhibits Immunosuppressive Activity of Myeloid-Derived Suppressor Cells in a TLR3-Dependent Manner. *J Immunol* 2018;200:2987–99.
8 Salameh ZS, Aycock KN, Alinezhadbalalami N, *et al*. Harnessing the Electrochemical Effects of Electroporation-Based Therapies to Enhance Anti-tumor Immune Responses. *Ann Biomed Eng* 2024;52:48–56.
9 Geboers B, Timmer FEF, Ruarus AH, *et al*. Irreversible Electroporation and Nivolumab Combined with Intratumoral Administration of a Toll-Like Receptor Ligand, as a Means of In Vivo Vaccination for Metastatic Pancreatic Ductal Adenocarcinoma (PANFIRE-III). A Phase-I Study Protocol. *Cancers (Basel)* 2021;13:3902.
10 Hemminki O, dos Santos JM, Hemminki A. Oncolytic viruses for cancer immunotherapy. *J Hematol Oncol* 2020;13.