



RESEARCH ARTICLE

Nanosecond Pulsed Field Ablation in Oncology

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ABSTRACT

Cancer therapy has evolved significantly over the past three decades and this review focuses on some of the new ablation modalities that have been developed as well as the importance of combination therapies which enlist the immune system. During the late 1990's various immune system checkpoints were discovered, and inhibitors of those immune checkpoints were found to boost the immune system enough in some patients to eliminate metastases. This revealed the power of the immune system to attack and eliminate tumors. However, for most patients simply inhibiting the immune checkpoints was insufficient and additional therapies were required. Current approaches involve ablation of the primary tumor followed by stimulation of the immune system to pick up tumor antigens released by the ablation and generate cytotoxic CD8+ T-cells to eliminate metastases. Tumor ablation involves the targeted delivery of either thermal or nonthermal energy sufficient to kill the tumor cells. Thermal ablation modalities were the first to be used but recent data suggest that the immune system is more strongly recruited by nonthermal modalities which have the additional advantage of sparing acellular structures such as neighboring nerves and blood vessels. We reviewed two studies that compared the efficacy of thermal with that of nonthermal ablation and concentrate on our progress using nanosecond pulsed field ablation. Recent studies that combine ablation with the injection of immune stimulants have shown that combination therapies are the most effective. Combining nsPFA with the intratumoral injection of immune stimulants boosts the immune system sufficiently to eliminate untreated tumors or metastases. We review the immune stimulants currently being used in preclinical studies and summarize the two clinical studies conducted thus far using nanosecond pulsed field ablation (nsPFA).

Introduction

Cancer therapy has improved dramatically over the past three decades in part due to improved surgical, radiation and ablation techniques, but more importantly due to advances in our understanding of the critical role of the immune system. This began with the discovery of immune checkpoint inhibitors¹ in the late 1990's and their amazing ability to completely eliminate cancer in a small percentage of patients². This demonstrated the importance of recruiting the immune system as an important component of any successful therapy in order to lead in the attack of tumor metastases. Specifically, the combination therapy of ablating the primary tumor followed by stimulation of the immune response is becoming a popular approach. This review will briefly describe both the commonly used ablation therapies and the many ways that the immune system can be stimulated following ablation of the primary tumor. nsPFA is the newest ablation therapy and will be the most thoroughly discussed.

Methods Used

A search of PubMed.gov was conducted in July, 2025 using keywords "tumor ablation" and "immune response" to identify combination studies using ablation techniques as well as the stimulation the immune system.

Tumor Ablation Technologies

Tumor ablation technologies fall into two main categories: thermal and nonthermal. Thermal mechanisms have been used for decades and include radiofrequency ablation (RFA), cryoablation, high intensity focused ultrasound (HIFU), hyperthermia (HT), and photothermal therapy (PTT). All of these thermal modalities generate sufficient temperature transitions to disrupt protein structure and damage cell membranes. Not only does this kill the treated cells but the spreading heat can damage nearby structures such as neighboring nerves and blood vessels. Two reviews of these thermal ablation modalities have appeared recently so they will not be considered in detail here^{3, 4}.

Nonthermal ablation mechanisms employ either pulsed electric fields to insert pores in cell membranes (electroporation) or short ultrasound bursts to mechanically disrupt cells (histotripsy^{5, 6}). Pulsed electric fields can have a range of cellular effects depending on their pulse duration and amplitude. The first pulsed fields applied to cells were in the microsecond range with amplitudes of 1-2 kV/cm⁷. When the pulse amplitude was below 2 kV/cm, the induced membrane pores and increased membrane permeability were transient and could be routinely used to load extracellular molecules into cells. However, when the pulse amplitude was increased to 2.5-3 kV/cm, the pores became permanent leading to the term, "irreversible electroporation" or IRE.

IRREVERSIBLE ELECTROPORATION (IRE)

This permanent electroporation ablates cells by necrosis⁸ and is available as the NanoKnife marketed by Angiodynamics. It has been approved by the FDA for ablating soft tissue and has been used in many clinical trials treating tumors⁹⁻¹¹. The initial therapy applied between 8 and 90 pulses 100 μ s in duration which required the application of paralytics due to the strong muscle contraction stimulated. More recently, shorter, high frequency biphasic signals have been used to reduce the muscle contractions and avoid paralytics.^{12, 13}.

HIGH FREQUENCY IRREVERSIBLE ELECTROPORATION (HFIRE)

These high frequency, biphasic pulses are typically composed of many one microsecond pulse pairs (positive followed by negative) strung together one after the other into packets of hundreds of pulse pairs. The biphasic nature reduces the strength of the muscle contractions triggered by IRE pulses, but these pulses are often delivered during the refractory period of the heart to avoid interfering with the normal cardiac rhythm. While not considered HFIRE, one company using biphasic waveforms is the Aliya platform marketed by Galvanize Therapeutics.

NANOSECOND PULSED FIELD ABLATION (nsPFA)

The most recent type of pulsed field ablation uses much shorter pulses in the nanosecond range (nsPFA) with much higher amplitudes. This technology is available as the nPulse™ marketed by Pulse Biosciences, Inc. nsPFA ablates cells using a different ablation mechanism. Rather than generating permanent pores that kill cells by necrosis, these shorter pulses are able to penetrate into the cell and are strong enough to generate smaller, transient pores¹⁴ in both the plasma membrane and the smaller organelle membranes¹⁵. This increases organelle permeability and disrupts the mitochondrial membrane potential, releasing cytochrome C¹⁶ and initiating the unique pathway of regulated cell death that is used by all cells when they reach the end of their useful life¹⁷. nsPFA can therefore be thought of as a catalyst that triggers the cell's natural cell death pathway rather than introducing sufficient energy to introduce permanent pores in cell membranes.

Histotripsy

The third modality for nonthermal ablation is histotripsy^{5,6}. Histotripsy uses a nonthermal modification of HIFU which applies the ultrasound waves in short bursts that mechanically vibrate the target cells to liquify them without significant heating. It has been approved by the FDA for hepatic targets and the first clinical trial treating hepatocellular carcinoma in 44 patients with liver tumors smaller than 1.5 cm demonstrated a primary efficacy of 95.5% with minimal complications (6.8%)^{18,19}. This technology is being marketed by HistoSonics Corp. Preclinical studies treating murine melanoma indicated a CD8+-dependent inhibition of tumor growth along with intratumoral infiltration of CXCR3+/CD8+ T-cells²⁰. Studies using two tumors in murine models indicated that treatment of one tumor with histotripsy slowed the growth of the untreated abscopal tumor²¹. There is a substantial literature comprised of over 400 papers describing the effectiveness of this nonthermal ablation modality which will not be covered any further in this review which will concentrate on the shorter electric pulses in the nanosecond domain and their use in oncology.

Studies comparing thermal ablation with nonthermal nsPFA

Two studies have been conducted to compare thermal ablation modalities with nonthermal nanosecond pulsed field ablation (nsPFA). The first of these compared the responses of human skin to nsPFA, radiofrequency ablation (RFA) and cryoablation²² (Fig. 1) and the second study compared the efficacies of nsPFA and cryoablation on eliminating murine melanoma tumors²³. nsPFA triggers regulated cell death as indicated by the appearance of activated Caspase-3 at 2 h post treatment along with the absence of nuclear staining 1 day post treatment (Fig. 2). Epidermal regeneration follows without impacting the acellular dermis in contrast to both cryoablation and RFA. RFA was found to trigger necrosis and cause scarring, inflammation or permanent pigmentary changes. The main differences between nsPFA and the other ablation modalities are the level of fibrosis, amount of scarring, elastic fiber concentration and inflammation. An analysis of the skin thickness 30 days after the treatments indicated that nsPFA-treated skin was most similar to untreated skin while cryoablated and RF-ablated skin were 2- and 3.5-fold thicker, respectively, suggesting that they induce inflammatory wound-healing processes that result in a substantially altered dermal layer.

The second comparative study contrasted cryoablation with nsPFA in the treatment of murine B16-F10 melanoma tumors²³. nsPFA permanently eliminated up to 91% of all tumor lesions with a single treatment compared to cryoablation which eliminated only 66% (Fig. 3). In addition, nsPFA exhibited much lower dermal fibrosis, underlying muscle atrophy and hair loss.

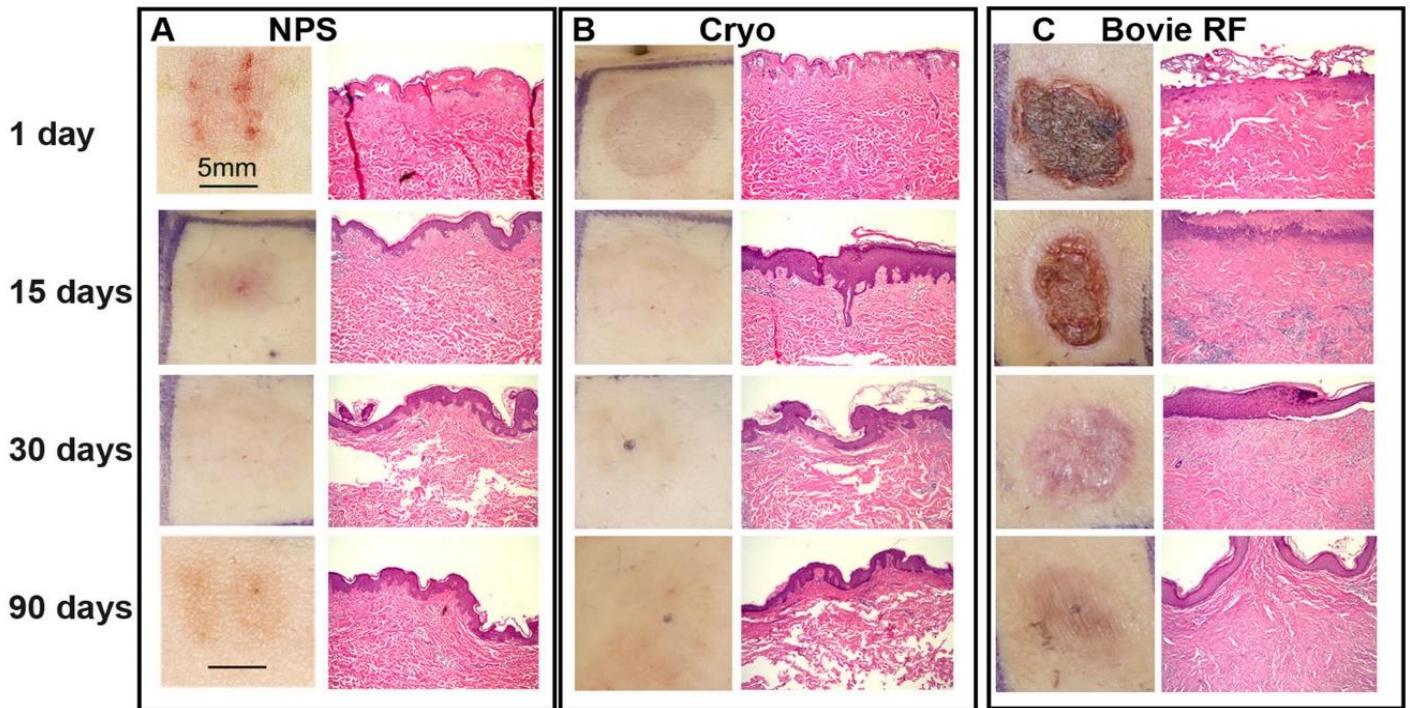


Figure 1. Surface photograph and corresponding histological section collected from human abdominal skin treated with the method indicated at the top of each column at the time point shown on the left. (A) NPS therapy applied at 130 mJ/mm^3 with a 5 X 5 mm tip; (B) Three seconds of cryoablation; (C) A few seconds of Bovie RFA ablation on a Bovie setting of 10. Scale bars on the upper left apply to all similar image types. (reprinted with permission²²)

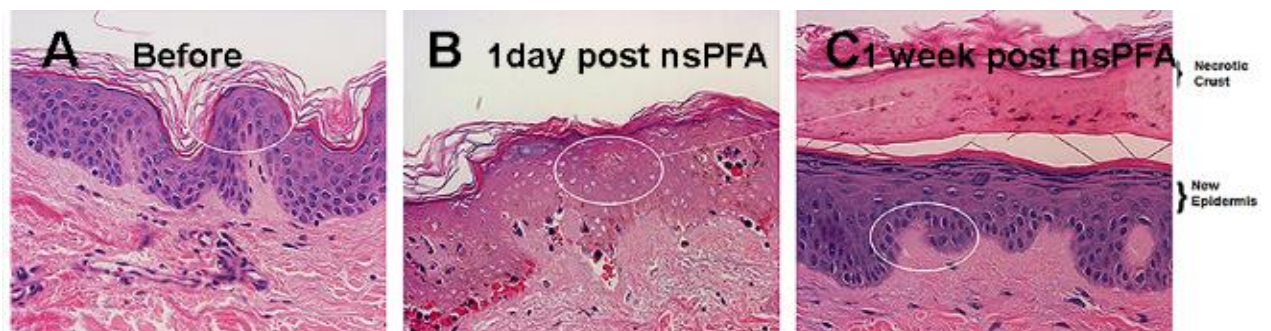


Figure 2. Time course of human epidermal viability loss and recovery. A. Thin section of untreated human skin stained with H&E; B. One day after nsPFA treatment; C. One week after nsPFA treatment; necrotic epidermis from “B” has the appearance of a crust and is delaminating from the new epidermal layer (oval). (reprinted with permission⁸²)

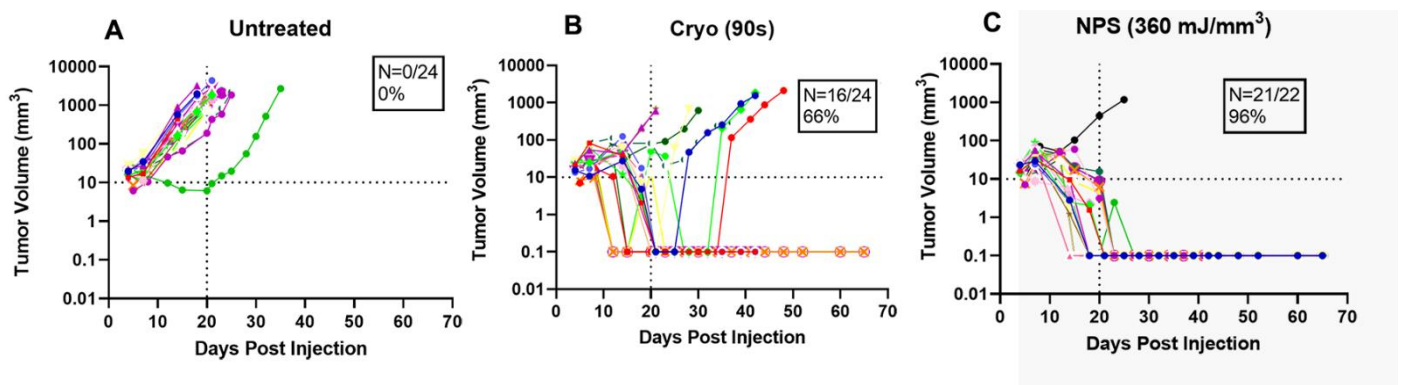


Figure 3. Melanoma tumor growth rates under the stated conditions. A. Untreated control tumor; B. Tumor treated with Cryo for 90s; C. Tumor treated with 360 mJ/mm^3 nsPFA. (reprinted with permission²³)

Unique Nature of Nanosecond Pulses

nsPFA has the unique ability to permeabilize intracellular organelle membranes because of the short duration and high amplitude of the fields applied. It is well known that biological lipid membranes can be permeabilized by applying a pulsed electric field of 400-500 mV/membrane. For a sub-micron organelle, that requires fields on the order of 1 volt across the diameter of 0.5 μm less which comes to a field strength of 20 kV/cm or greater. Applying such a large field for a duration of microseconds or milliseconds would heat the cell beyond hyperthermia levels. However, the much shorter delivery time of nsPFA avoids this heating and allows a nonthermal energy delivery that generates 1 nm-wide pores in both plasma and organelle membranes while leaving these membranes intact. This nsPFA thereby disrupts mitochondrial function and initiates regulated cell death (RCD)²⁴. RCD is the death process used by all cells at the end of their useful life. This process is initiated by cellular signals indicating that something has gone wrong, such as a DNA mutation or membrane damage that the cell cannot repair. These cells signal the dendritic cells of the immune system to phagocytose them and incorporate all the tumor antigens. Sharing these antigens with T-cells in the lymph nodes leads to the activation of cytotoxic T-cells specific for cells displaying those tumor antigens.

Evidence that nsPFA initiates RCD

The RCD mechanism has several well-studied components that have been observed following nsPFA. The first is the release of signaling molecules called, "DAMPs", which stands for danger-associated molecular patterns. These signaling molecules include ATP, HMGB1 and calreticulin and attract dendritic cells to the treated region to phagocytose the dying cells. nsPFA treatment has been shown to lead to the release of these DAMP molecules²⁵. In fact, a recent study of the DAMPs released by nanosecond, microsecond and millisecond pulse widths found that nsPFA was the only pulse width that released all three of the DAMP molecules²⁶. ATP and HMGB1

serve as chemo-attractants for dendritic cells such as macrophages. Calreticulin is a protein normally resident in the endoplasmic reticulum, but it is translocated to the plasma membrane during RCD²⁷. There it can bind to CD40L on dendritic cells and promote their maturation, initiating phagocytosis that incorporates tumor antigens. If any of the antigens are foreign, the immune system will generate cytotoxic CD8+ T-cells which will circulate in the body and attack any cells expressing those foreign antigens.

Evidence that nsPFA treatment can result in CD8+ cytotoxic T-cell production

The earliest indication that nsPFA tumor treatment resulted in CD8+ T-cell production came from attempts to rechallenge mice several weeks after the primary tumor had been eliminated by nsPFA. The injection of the same line of some tumor cells failed to generate another tumor (Fig. 4). Further studies on a variety of tumor types indicated that the strength of this blockage of rechallenge tumor growth varied with the tumor type²⁸ suggesting that there are varying amounts of CD8+ T-cell production.

Further evidence for the production of CD8+ T-cells came from immunohistochemistry labeling of the untreated, abscopal tumors (Fig. 5). Labeling thin sections of untreated tumors in mice for which the primary tumor had been either ablated with nsPFA or resected indicated much more CD8+ infiltration in those untreated tumors in mice whose primary tumor had been ablated²⁹. In addition, this increase was reduced when CD8+ antibodies were introduced providing more evidence that these were indeed CD8+ T-cells (Fig. 5C).

Perhaps the strongest data in support of tumor-specific CD8+ T-cell production comes from abscopal studies in which two tumors are injected into the same mouse and only one is ablated. By following the changes in the growth of the untreated tumor, one can determine if CD8+ T-cells are attacking it. We recently completed some abscopal studies of

pancreatic tumors (Fig. 6). In order to strengthen the immune response, we injected an immune stimulant, aOX40, into the nsPFA-treated tumor immediately after treatment. We observed that the combination of nsPFA treatment plus aOX40 injection completely eliminated the primary tumor and also eliminated the abscopal tumor in 80% of

the mice. These data suggest that we had elicited an immune response that would eliminate metastases which is the ultimate goal. The use of immune stimulants is becoming more common as we find that ablation modalities that generate DAMPs do not normally sufficiently stimulate the immune response to completely eliminate metastases.

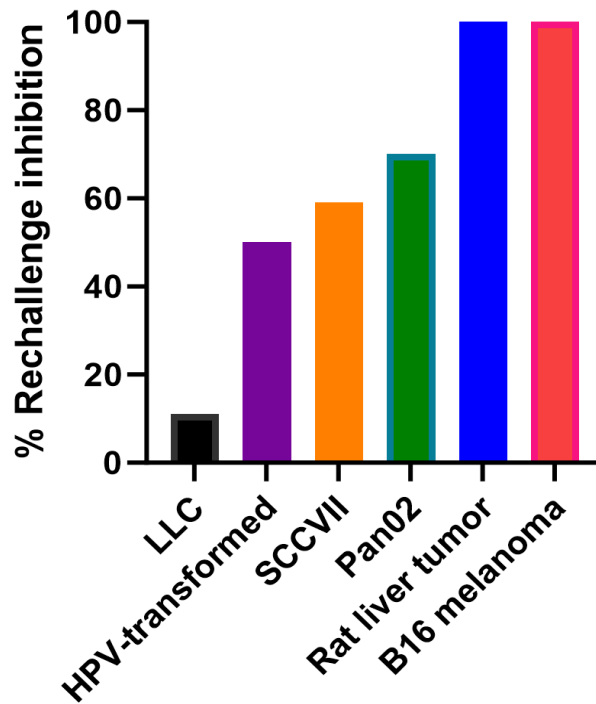


Figure 4. Rechallenge tumor inhibition varies with tumor type, suggesting varying amounts of CD8+ T-cell production (reprinted with permission²⁸)

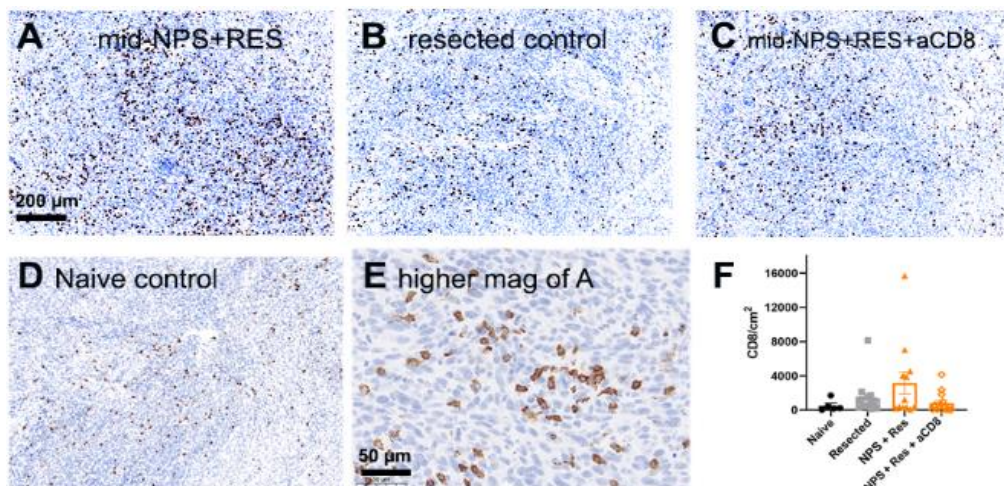


Figure 5. Immunohistochemistry of rechallenge tumors labeled with anti-CD8 antibodies (brown) 30 days postinjection. (A) Rechallenge tumor taken from mouse whose primary tumor was treated with mid-range NPS + resiquimod (RES) injection immediately afterward (15,690 CD8/mm²). (B) Rechallenge tumor taken from mouse whose primary tumor was resected (2140 CD8/mm²). (C) Rechallenge tumor taken from mouse whose primary tumor was treated with mid-range NPS + RES with anti-CD8 antibodies injected IP into the mouse (4140 CD8/mm²). (D) Tumor taken from mouse who did not receive a prior injection of Pan02 cells (Naive) (180 CD8/mm²). (E) Higher magnification of a section from 3A. (F) Plot of the distribution of CD8 densities in four conditions indicated. Error bars represent SEM. (reprinted with permission²⁹).

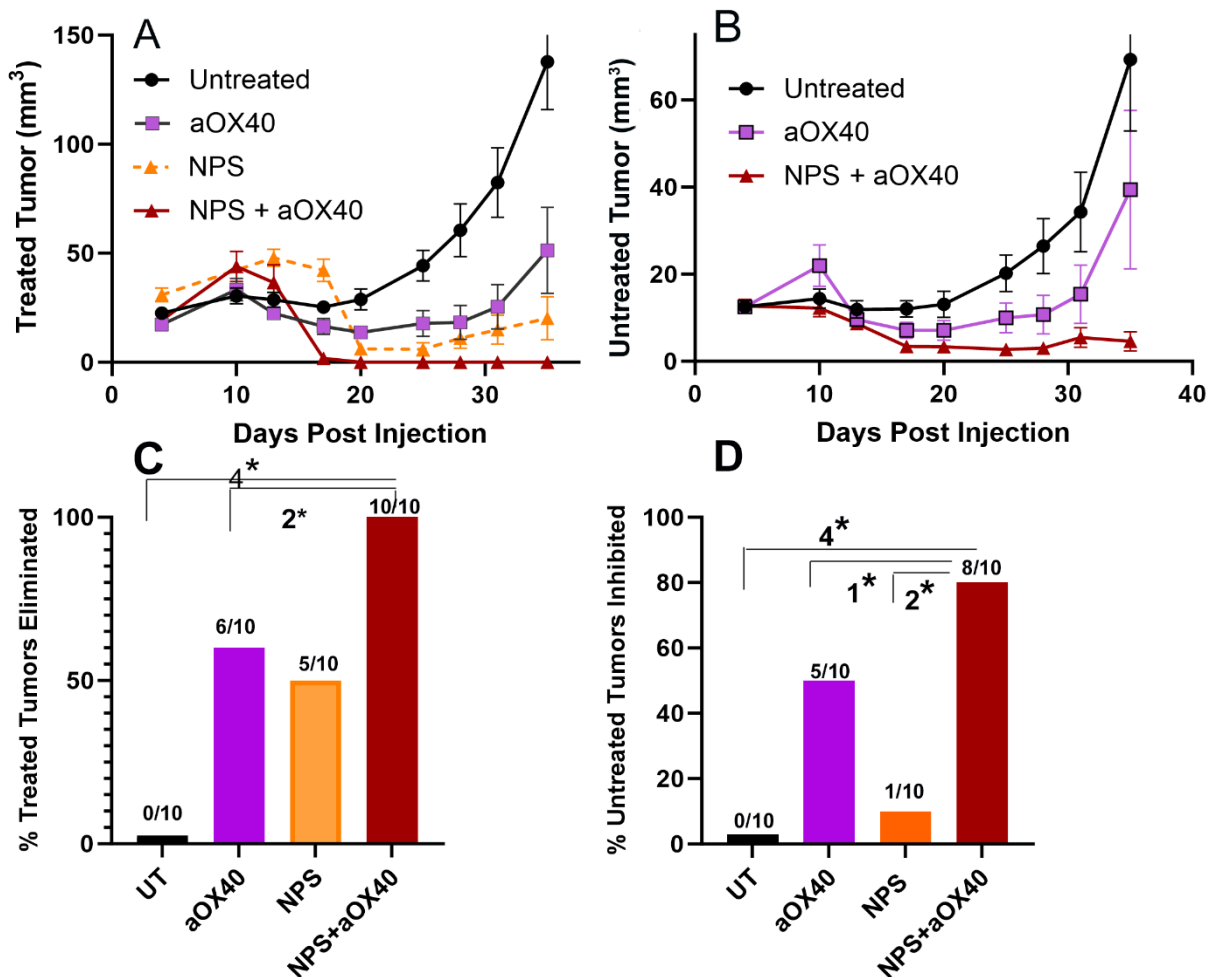


Figure 6. Treated and untreated tumor growth after treating the primary tumor with either NPS (180 mJ/mm³), aOX40 i.t. injection, or the combination of both. (A) Time course of treated tumor growth when the primary tumor was either untreated or treated with NPS (180 mJ/mm³), aOX40, or the combination of both. When aOX40 was combined with NPS the treated tumor growth was more strongly inhibited than with either alone. (B) Time course of untreated (abscopal) tumor growth following injection of the primary tumor with either 50 µg aOX40 or the combination of NPS treatment followed by 50 mg aOX40 i.t. injection. The combination treatment exhibited the strongest inhibition of untreated tumor growth. Error bars in A and B represent SEM. (C) Percentage of treated tumors eliminated by day 30. (D) Percentage of untreated abscopal tumors inhibited by the indicated treatment of the primary tumor (4**p* < 0.0001, 3**p* < 0.001, 2**p* < 0.01, 1**p* < 0.05). (Reprinted with permission²⁹)

Immune Stimulants Used in Combination with Ablation Modalities

A survey of the literature identified several immune stimulants used in combination with various ablation modalities to enhance the immune system's ability to eliminate untreated tumors:

aOX40 and CpG: OX40, also known as CD134, is a co-stimulatory receptor on the surface of activated T-cells that belongs to the TNF receptor superfamily. It plays a role in T-cell activation, proliferation and survival. OX40 agonists, such as ivuxolimab or aOX40, can enhance the function of tumor-reactive

T-cells and promote the anti-tumor immune response. In the work cited above (Fig. 6) we injected aOX40 intratumorally after nsPFA to eliminate untreated pancreatic abscopal tumors²⁹. One of the pioneering studies using intratumoral injection of aOX40 combined with the synthetic toll-like receptor TLR9 ligand, *CpG*, eradicated untreated sites of murine colon carcinoma and breast cancer³⁰. Unfortunately this therapy was not as successful when used in a clinical trial³¹.

Imiquimod or Resiquimod: stimulates the innate immune system by activating toll-like receptor 7 (TLR7), commonly involved in pathogen recognition. Cells activated by imiquimod secrete cytokines such as interferon- α , interleukin-6 and tumor necrosis factor. Intratumoral injection of a gel form of imiquimod combined with aPD-1 rescued mice after colon carcinoma treatment with cryoablation³².

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Bacillus Calmette-Guerin (BCG): GM-CSF is a cytokine that plays a crucial role in the development and function of white blood cells, particularly granulocytes and macrophages. BCG is a weakened form of the bacterium *Mycobacterium bovis* that is primarily used as a vaccine against tuberculosis and also in the treatment of recurrent bladder cancer³³. The combination of RFA on the primary tumor and GM-CSF/BCG injection completely eliminated distant mouse liver tumors³⁴. Similarly, injection of GM-CSF and purified protein derivatives (from BCG) eliminated the abscopal tumors in a murine colon carcinoma model³⁵.

CD40: a transmembrane protein found on antigen-presenting cells that is required for their activation. When CD154 binds to CD40, it activates the antigen-presenting cells. Addition of the CD40 agonist, aCD40, to IRE-treated murine pancreatic tumors improved dendritic cell activation and generated a

strong systemic antitumor T-cell response that inhibited metastatic disease progression³⁶.

OK432: a freeze-dried product of streptococcus pyogenes plus penicillin, also known as *Picibanil*, induces an inflammatory response. When the injection of OK-432 followed RFA treatment of osteosarcoma, distant untreated tumors shrink in size³⁷.

Ablating Murine Tumors with nsPFA

We have developed a very simple method for generating tumors in mice and treating them with nsPFA. When tumor cells are injected intradermally, the tumors will develop within the mouse skin which can be stretched over a light post for easy imaging of the tumor outline (Fig. 7). We use a row of electrodes on either side of the tumor to expose all the tumor cells to our energy and the holes formed by the electrode penetration indicate the boundary of the applied field. Using this approach we have treated a wide range of murine tumor types with several field strengths, including B16 melanoma³⁸⁻⁴², 4T1 breast tumors²⁸, LLC lung carcinomas (Fig. 8), SCCVII squamous cell carcinomas²⁸, and Pan02 pancreatic tumors²⁹. We found that the nsPFA energy required to eliminate these tumors varies with tumor type (Table 1). Several other labs have been using nsPFA to treat murine tumors and I have referenced those published over the past 6 years^{24, 43-77}.

Table 1. Energy dependence of tumor ablation (reprinted with permission ²⁸)	
Murine Tumor Type	Energy for 90% Ablation (mJ/mm ³)
Pancreatic carcinoma	240
Lung carcinoma	240
Breast carcinoma	360
Squamous cell carcinoma	360
Melanoma	480

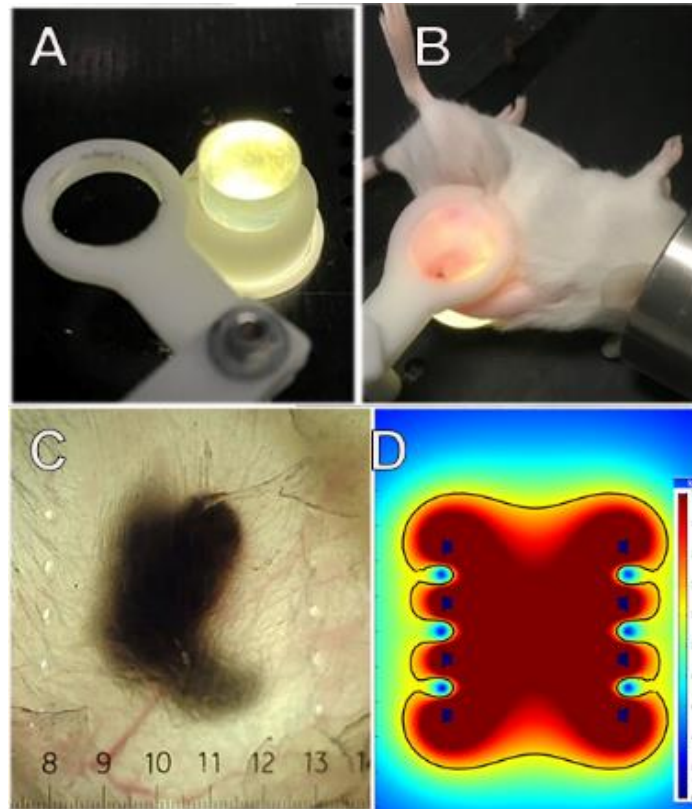


Figure 7. Method for treating the entire murine tumor. A. Photo of lighted silicone post over which the skin encapsulating the tumor is stretched. The white ring is placed over the skin to hold it once the tumor is in position. B. Photo of mouse with skin stretched over post for transillumination; C. Image of a melanoma tumor after treatment with two rows of bipolar needle electrodes. Holes indicate position of the two rows of electrodes; D. COMSOL Multiphysics model of the electric field distribution between the two rows of needle electrodes (black dots) indicating the uniformity of the applied electric field. (Reprinted with permission²⁸)

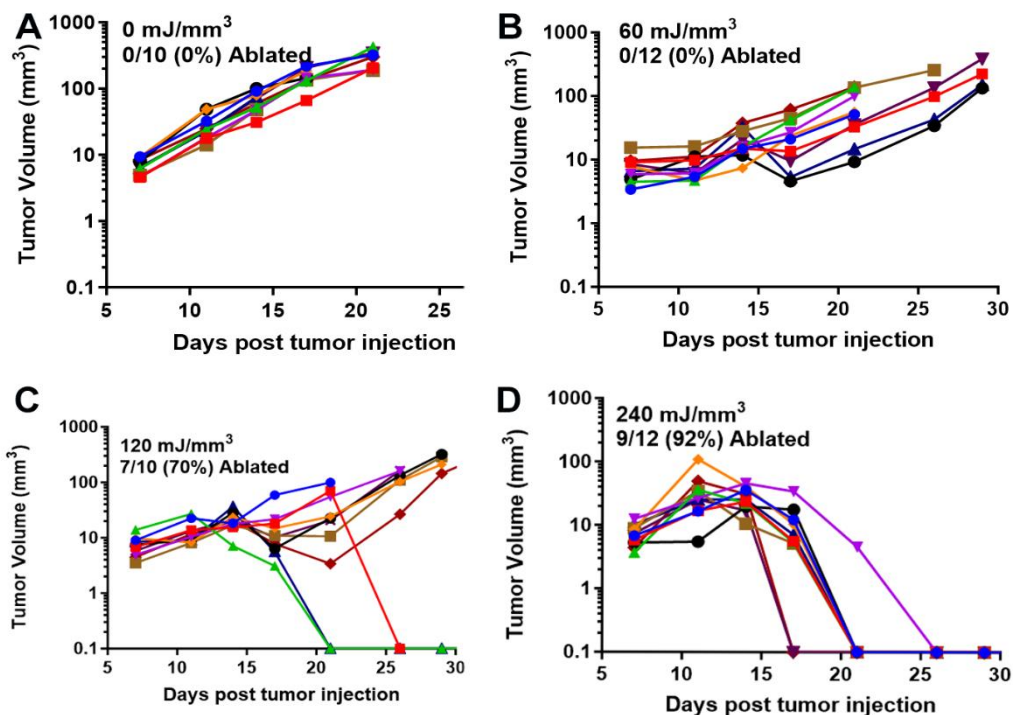


Figure 8. Growth of Lewis Lung carcinomas injected on Day 0 and treated on Day 5 with the indicated NPS energy. A. In the Sham control, none of the 10 treated tumors were ablated; B. 60 mJ/mm³ treatment ablates none of the 12 treated tumors; C. 120 mJ/mm³ ablates three of the 10 tumors treated; D. 240 mJ/mm³ treatment ablated all 10 of the 10 treated tumors. (reprinted with permission²⁸)

Clinical Studies

Despite the large number of studies demonstrating success in using nsPFA to treat murine tumors, there have been very few clinical trials applying nsPFA to human tumors. Up to now, only two human oncology clinical trials have been published, basal cell carcinoma and hepatocellular carcinoma.

BASAL CELL CARCINOMA

The first oncology clinical trial using NPS therapy treated 10 basal cell carcinomas (BCC) on only three subjects⁷⁸. Seven of the treated lesions were completely free of basaloid cells when biopsied, but 2 of the 7 exhibited seborrheic keratosis in the absence of basaloid cells. No scar was visible on the healed sites of any of the successfully ablated lesions. More recently, a larger trial treated 37 BCC lesions on 30 subjects, and 92% showed complete histological clearance of BCC⁷⁹. Histological analysis of the three cases where residual BCC was noted indicated that full energy coverage was not achieved. This study concluded that these nanosecond pulses were safe and effective for clearing both nodular and superficial BCC lesions. This success treating the deeper, nodular BCCs is noteworthy because they are resistant to treatment with other modalities.

HEPATOCELLULAR CARCINOMA

The first clinical study to evaluate the efficacy and safety of NPS for the treatment of hepatocellular carcinoma (HCC) began in 2020 in five hospitals in China and treated 192 patients with HCC ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04309747), NCT04309747)^{71, 80, 81}. These HCC tumors were in high-risk locations where thermal ablation was not advised due to nearby blood vessels or biliary ducts. After contrast-enhanced magnetic resonance imaging, three-dimensional tumor measurements were used to determine the required electrode number and configuration. Two-six 19-gauge unipolar nsPEF electrodes were inserted percutaneously in a parallel manner with ultrasound or CD guidance. They had an interelectrode distance of 10-25 mm and active tip length of 20 mm. 800

pulses 300ns long and 25-30 kV/cm in amplitude were applied. The complete ablation rate at 1 month was 86% (90% for tumors smaller than 2 cm and 72% for tumors larger than 2 cm) with a low incidence of high-grade AEs.

Conclusion

We have concentrated here on the nsPFA nonthermal ablation modality due to its enhanced safety and cellular-specific targeting which avoids damaging acellular structures. Pulsed electric fields vary mainly by pulse width and amplitude as well as the type of cell death that they initiate. It is only the ultrashort nanosecond pulses that can be applied at amplitudes large enough to permeabilize the small intracellular organelles and initiate RCD rather than necrosis. Initiating RCD has the advantage of recruiting the immune system to generate cytotoxic CD8+ T-cells to attack any cells expressing tumor antigens. While the immune response triggered by nsPEF alone is not usually sufficient to eliminate untreated tumor cells, the addition of immune system stimulants such as aOX40 can strengthen the immune response sufficiently to eliminate untreated tumor cells, raising the hope that metastases might be eliminated when nsPFA is combined with immune stimulants. While the clinical work applying nsPFA is in its infancy, the preliminary results are quite encouraging and suggest that high efficacy should be possible. The application of nonthermal nsPFA along with immune stimulants will be a major focus for oncology research in the coming years.

Conflict of Interest Statement:

R. Nuccitelli is employed by Pulse Biosciences, Inc.

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None

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None

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