Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 5 Issue 8 August 2021

Mini Review

Nanopulse Stimulation as a Possible Immunotherapy

Stephen J Beebe*

Frank Reidy Research Center for Bioelectrics, Old Dominion University, Virginia, USA

***Corresponding Author:** Stephen J Beebe, Frank Reidy Research Center for Bioelectrics, Old Dominion University, Virginia, USA.

Received: April 15, 2021

Published: July 08, 2021

Abstract

Nanopulse stimulation (NPS) presents a novel approach for cancer treatment. It applies high electric field pulses with ultrashort durations and fast rise-fall times, affecting the plasma membrane and intracellular structures and functions, inducing regulated cell death mechanisms that are immunogenic, eliminating tumors and their immunosuppressive tumor microenvironment, and activating innate and adaptive immune mechanisms. This results in tumor ablation and *in situ* vaccination against rat liver and mouse breast cancer. Here I provide a general overview of this technology and some finer points of the pulsed power technology.

Keywords: Pulsed Power; Tumor Ablation; *In Situ* Vaccination; Immunosuppression; Innate Immunity; Adaptive Immunity

Nanopulse stimulation (NPS), a pulsed power technology

The application of nanopulse stimulation (NPS), also called nanosecond pulsed electric fields (nsPEFs) and nanosecond electric pulses (nsEP), is a unique and novel strategy for cancer therapy [1-3]. NPS was established from pulsed power technology borrowed from high-powered physics. For example, pulsed power releases 1 joule of stored electrical energy in a nanosecond providing 1 gigawatt of power, vs. releasing that stored energy in a second, providing 1 watt of power. So, compressing the electrical energy and releasing it instantly provides high-power non-thermal electrical pulses. A most successful NPS treatment strategy for cancer ablation and inducing an immune response applies one thousand 100 ns duration pulses (10 megawatts/pulse), with fast (short) rise-fall times (5 - 10 ns) with 3 pulses per second (3 Hz), and an electric field of 50 kV/cm. This delivers a charging impact of 5 Vs/cm (Eτn: E = electric fields in volts/cm x τ = pulse duration in sec, x n= pulse number). Intracellular effects occur during the short (fast) rise and fall times of nanosecond pulses coinciding with high-frequency components, while plasma membrane effects occur during the pulse plateau.

Pulses with short durations and fast(short) rise-fall times induce intracellular effects

Pulses with short duration and fast (short) rise-fall times summon the plasma membrane charging time constant, or perhaps more fitting, the inverse of that constant in the frequency domain {Hz = 1/time (s)} [4-6]. Thus, the pulse duration and rise-fall time should be shorter (or faster) than the time for the redistribution of charges over the plasma membrane that would prevent electric fields from entering the cell [5,6]. For cells in suspension, the charging time constant is about 75 ns [5-7]; however, that constant is much longer for cells in tumors, perhaps ~1 μs. In both cases, the plasma membrane becomes more transparent for electric fields changing in shorter time frames than the charging time constant [8]. Thus, short pulse durations and short rise-fall times can be considered better for immunity because of frequency considerations with broader high-frequency content. This has advantages because it favors electric field penetration through the cell's capacitive branch in an equivalent circuit.

The concept for short pulse duration that affects intracellular membranes or mechanisms is supported by the finding that shorter pulse durations with fast rise-fall times were superior to longer pulse durations with fast rise-fall times for the release of Ca^{2+} from the endoplasmic reticulum [9]. In addition, support for the importance of the rise-fall time concept was supported by the finding that a single pulse with 15 ns rise-fall time was superior to pulses with 150 ns rise-fall time for dissipation of the ΔΨm and RCD in liver cancer cells [10,11].

NPS induces intracellular effects that can be immunogenic

NPS-induced intracellular effects are received by intracellular membranes like ER and mitochondrial membranes; however, other less well-characterized structures are also affected. Possibly, NPS can alter the conformation or structure of proteins shown by inactivation of the PKA catalytic subunit's catalytic function and thereby modifying g the structure of the enzyme (structure = function) [12]. So, what RCD mechanisms are induced in response to NPS, and what reactions do they elicit? A hypothesis is that the mechanism of RCD is crucial because it does or does not produce molecules with damage-associated molecular patterns (DAMPs) or alarmins. These include calreticulin, ATP, HMGB1, among others that activate the innate immune system, cause inflammation, and mature and activate antigen-presenting cells (APC), such as dendritic cells (DCs) [13,14]. Thus, some mechanisms that induce RCD and release DAMPs will cause immunogenic cell death (ICD) and immunity.

Because ICD factors, such as calreticulin (ER), HMGB1 (nucleus), and ATP (mitochondria and cytoplasm), are released from the intracellular environment, it is reasonable that short pulse durations and rise-fall time that affect the intracellular environment should enhance the release of ICD factors and to improve antigen recognition for enhanced immunity. For example, NPS released ICD factors from 4T1-luc BC cells when pulsed with lower NPS conditions [15], which occur on the NPS-treated fringes TME. This was also observed in other cell types [16].

The release of calreticulin, ATP, and HMGB1 from 4T1-luc cells was predicted based on the finding that NPS induced immune-mediated vaccine effects in these mammary cancer tumors that amounted to an *in situ* vaccination. There were several NPS-induced responses from the TME and the host immune system that characterized this immunity. Before discussing NPS-induced immunome responses, let's consider the challenges the TME presents to NPS and other therapies.

A complex and hostile TME

There are many realized obstructions for cancer therapeutics. These obstacles include the enormous numbers of cancer-causing mutations [17], overlaps among classical cancer hallmarks [18], genomic instability, structural and temporal clonal heterogeneity [19,20], cancer stem cells [21-23], epigenetic modifications [24-26], cancer evolution [27-31] and genome-centric views, which include stochastic changes, such as chromosomal reorganization, copy number variations, gene duplications, and non-clonal chromosomal aberrations, as opposed to gene-centric outlooks [32,33].

While cancer hallmarks [34,35] and driver genes [17] underlie many of these cancer mechanisms, their compounded complexities present cancer in a problematic light regarding targeted cancer therapy. Intratumoral heterogeneity most often requires a need for multiple therapies. Combination therapy has historically been a foundation of cancer treatments as drugs are used in succession, usually when drug resistance arises with the initial treatment. More recently, combination therapies are being used at the outset of therapy, and some may provide synergistic effects [36,37]. However, tumor heterogeneity can generally normalize drug combination improvements, so the unique benefit of a particular drug on a specific subpopulation of cancer cells can sometimes be reduced [20].

Tumors most often harbor sub-populations of clones with distinct genotypes, epi-genotype, karyotypes, and phenotypes that span several cancer hallmarks, exhibit different behaviors and metabolisms and present different therapeutic sensitivities and resistances. Malignant transformations begin as multiple DNA mutations in oncogenes and/or tumor suppressor genes. Tolerance for DNA transcription errors leads to unstable genomes. This allows clonal heterogeneity and expression of different diseases with different sensitivities to chemotherapeutic drugs and targeting treatments. With a focus on killing cancer diseases, a targeted drug eliminates a specific clone, a chemotherapeutic agent may kill several clones. Still, these approaches leave niches for the growth of a resistant clone (s) requiring a different drug (s) for clearance. In contrast, immunotherapy focuses on unleashing the host's immune system.

NPS as a unique immunotherapy

As determined so far, NPS is a cancer immunotherapy that includes two distinct therapeutic strategies. It is generally a targeted ablative treatment; however, not in the sense of a clonal-specific agent, a chemotherapeutic agent, or a protein kinase inhibitor. Instead, it assaults the entire tumor mass within the electrodes, including immunosuppressive cells and corrupted host supportive cells in the TME. It disrupts the TME irrespective of what kind of cancer it carries, regardless of unstable genomes, specific cancer mutations, molecular activated pathways, cancer stem cells, or clonal heterogeneity. The TME is revolutionized by the charging effects of NPS ultrashort electric pulse duration with fast rise-fall time pulses that kill cancer cells by permeabilizing all cell membranes, affecting intracellular structures and functions. These conditions are suspected to be the mechanisms that lead to activation of the innate immune system, the release of immunogenic factors and cancer stress ligands, and the induction of RCD mechanisms that lead to enhanced antigen recognition and the ultimate establishment of immune memory.

The immunosuppressive TME

In addition to tumor heterogeneity and unstable genomes, the immunosuppressive TME is a most severe problem of a different nature. Cancer cells and altered host cells that promote tumorigenesis can apply many suppressive mechanisms against cancer therapies. This is a primary obstacle for immunotherapy. Activation of the host immune system can only be successful if the treatment resolves the immunosuppressed TME.

The disruption of the TME resolves the cancer control over the immunosuppressed TME and prevents immunoreactive mechanisms allowing positive immune responses. Fortunately, NPS has a prominent effect of eliminating the suppressive TME uses this advantage to activate innate and adaptive host immune responses. While these positive immunome responses occur in the mouse 4T1-luc and N1-S1 liver cancer model, it may be overly optimistic to consider this a typical response. In two orthotopic models, rat hepatocellular carcinoma (HCC) [38,39] and mouse breast cancer (BC) [15,40], animals were *in situ* vaccinated, making them resistant to the treated cancers.

NPS eliminates cells by RCD as delayed mechanisms that are definable and quantifiable *in vivo* [38,41-43]. We propose that NPS targets specific cell death mechanisms that more readily activates innate and adaptive immunity. First, NPS effectively eliminates orthotopic breast and liver tumors and releases immunogenic cell death (ICD) molecules, including increases in ATP, HMGBI, and calreticulin [15]. Second, NPS treated 4T1-luc mammary cancer cells upregulate dendritic cell (DC) activation markers CD40, MHCII [15], and cytokine/chemokine secretion [Beebe., *et al*. unpublished]. Third, NPS-treated liver tumors induce increases in specifically activated subsets of natural killer cells (NKs) and NKT-cells (NKTs) followed by increases in cytotoxic liver T-cells with multiple adaptive memory phenotypes [39]. NPS also eliminates immunosuppressive cells in the TME, dLN, spleen, and blood, allowing cytotoxic T-cells to kill tumor cells [15,39,40] and attenuate distant organ metastasis, as shown in the mouse breast cancer (BC) model [15]. However, rat liver and mouse BC cells do not die *in vitro* by apoptosis, nor do human triple-negative BC (TNBC) cells, which die by necroptosis and/or parthanatos [43]. The literature suggests many RCD mechanisms are immunogenic, including apoptosis, regulated necrosis, autophagy, or all of them [44-48]. Nevertheless, increasing evidence indicates that tumor RCD mechanisms enhance immune responses through ICD [46,49,50].

In contrast to the HCC and BC models, NPS did not induce immunity in the pancreatic cancer (PC) model due partly to the lingering presence of Tregs and MDSCs in the TME after NPS treatment [51]. Others also failed to induce immunity in B16f10 melanoma [52]. However, these studies used NPS with slower rise-fall times.

Future Considerations

While immunogenicity is generally defined by the cancer model, the finding that NPS may impact immunogenicity based on RCD effects and ICD determinants suggests some experimental latitude for NPS-induced immunity among tumor models. It may be necessary to combine NPS with other therapies, especially immunotherapies, to eliminate the immunosuppressive TME and induce immune responses.

Bibliography

1. Beebe SJ. "Preclinical Studies on Nanosecond Pulses". In Handbook of Electroporation, Miklavčič D., Ed.; Springer, Cham (2016): 1543-1562

2. Beebe SJ. "Regulated and Apoptotic Cell Death After Nanosecond Electroporation". In Handbook of Electroporation. Miklavčič D., Ed.; Springer, Cham. (2016): 511-528.

3. Beebe SJ. "Regulated Cell Death (RCD) by Caspase-Dependent Mechanisms". In Bioelectrics, Akiyama H., Heller R, Eds.; Springer Japan (2016): 238-254.

4. Schoenbach KH., *et al*. "The effect of pulsed electric fields on biological cells: Experiments and applications". *IEEE Trans on Plasma Science* 25 (1997): 284-292.

5. Schoenbach KH., *et al*. "Intracellular effect of ultrashort electrical pulses". *Bioelectromagnetics* 22 (2001): 440-448.

6. Schoenbac K H., *et al*. "Ultrashort electrical pulses open a new gateway into biological cells". *Proc. IEEE* 92 (2004): 1122-1137.

7. Cole KS. "Electric impedance of marine egg membranes". *Transactions of the Faraday Society* 23 (1937): 966-972.

8. Schwan HP. "Dielectric properties of the cell surface and electric field effects on cells". *Studia Biophysica* 110 (1985): 13-18.

9. Semenov I., *et al*. "Recruitment of the intracellular Ca2+ by ultrashort electric stimuli: the impact of pulse duration". *Cell Calcium* 54 (2013): 145-150.

10. Beebe SJ., *et al*. "Transient features in nanosecond pulsed electric fields differentially modulate mitochondria and viability". *PLoS One* 7 (2012): e51349.

11. Beebe SJ., *et al*. "Induction of Cell Death Mechanisms and Apoptosis by Nanosecond Pulsed Electric Fields (nsPEFs)". *Cells* 2 (2013): 136-162.

12. Beebe SJ. "Considering effects of nanosecond pulsed electric fields on proteins". *Bioelectrochemistry* 103 (2015): 52-59.

13. Keep O., *et al*. "Immunogenic cell death modalities and their impact on cancer treatment". *Apoptosis* 14 (2009): 364-375.

14. Galluzzi L., *et al*. "Consensus guidelines for the definition, detection, and interpretation of immunogenic cell death". *Journal of Immunotherapy Cancer* 8 (2020): e000337.

15. Guo S., *et al*. "Nano-pulse stimulation induces potent immune responses, eradicating local breast cancer while reducing distant metastases". *International Journal of Cancer* 142 (2018): 629-640.

16. Nuccitelli R., *et al*. "Nano-Pulse Stimulation is a physical modality that can trigger immunogenic tumor cell death". *Journal of Immunotherapy Cancer* 5 (2017): 32.

17. Vogelstein B., *et al*. "Cancer genome landscapes". *Science* 339 (2013): 1546-1558.

18. Horne SD., *et al*. "Stress, genomic adaptation, and the evolutionary trade-off". *Frontiers in Genetics* 5 (2014): 92.

19. Salk JJ., *et al*. "Mutational heterogeneity in human cancers: origin and consequences". *Annual Review of Pathology* 5 (2010): 51-75.

20. Zhao B., *et al*. "Intratumor heterogeneity alters most effective drugs in designed combinations". *Proceedings of the National Academy of Sciences of the United States of America* 111 (2014): 10773-10778.

21. Dean M., *et al*. "Tumour stem cells and drug resistance". *Nature Reviews Cancer* 5 (2005): 275-284.

22. Beck B and Blanpain C. "Unravelling cancer stem cell potential". *Nature Reviews Cancer* 13 (2013): 727-738.

23. Easwaran H., *et al*. "Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance". *Molecular Cell* 54 (2014): 716-727.

24. Bird A. "Perceptions of epigenetics". *Nature* 447 (2007): 396-398.

25. Waddington CH. "The epigenotype". *International Journal of Epidemiology* 41 (2012): 10-13.

26. Byler S., *et al*. "Genetic and epigenetic aspects of breast cancer progression and therapy". *Anticancer Research* 34 (2014): 1071-1077.

27. Nowell PC. "The clonal evolution of tumor cell populations". *Science* 194 (1976): 23-28.

28. Heng HH. "The genome-centric concept: resynthesis of evolutionary theory". *Bioessays* 31 (2009): 512-525.

29. Heng HH., *et al*. "The evolutionary mechanism of cancer". *Journal of Cellular Biochemistry* 109 (2010): 1072-1084.

30. Horne SD., *et al*. "Stress, genomic adaptation, and the evolutionary trade-off". *Frontiers in Genetics* 5 (2014): 92.

31. McGranahan N and Swanton C. "Biological and therapeutic impact of intratumor heterogeneity in cancer evolution". *Cancer Cell* 27 (2015): 15-26.

32. Heng HH., *et al*. "Cancer progression by non-clonal chromosome aberrations". *Journal of Cellular Biochemistry* 98 (2006): 1424-1435.

33. Heng HH. "The genome-centric concept: resynthesis of evolutionary theory". *Bioessays* 31 (2009): 512-525.

34. Hanahan D and Weinberg RA. "The hallmarks of cancer". *Cell* 100 (2000): 57-70.

35. Hanahan D and Weinberg RA. "Hallmarks of cancer: the next generation". *Cell* 144 (2011): 646-674.

36. Al-Lazikani B., *et al*. "Combinatorial drug therapy for cancer in the post-genomic era". *Nature Biotechnology* 30 (2012): 679-692.

37. Gatzka MV. "Targeted tumor therapy remixed-an update on the use of small-molecule drugs in combination therapies". *Cancers (Basel)* 10 (2018): 155.

38. Chen R., *et al*. "A protective effect after clearance of orthotopic rat hepatocellular carcinoma by nanosecond pulsed electric fields". *European Journal of Cancer* 50 (2014): 2705-2713.

39. Lassiter BP., *et al*. "Nano-Pulse Stimulation Ablates Orthotopic Rat Hepatocellular Carcinoma and Induces Innate and Adaptive Memory Immune Mechanisms that Prevent Recurrence". *Cancers (Basel)* 10 (2018): 69.

40. Beebe SJ., *et al*. "Nanopulse stimulation (NPS) induces tumor ablation and immunity in orthotopic 4T1 mouse breast cancer: A review". *Cancers (Basel)* 10 (2018): 97.

41. Chen X., *et al*. "Apoptosis initiation and angiogenesis inhibition: melanoma targets for nanosecond pulsed electric fields". *Pigment Cell and Melanoma Research* 23 (2010): 554-563.

42. Chen X., *et al*. "Long term survival of mice with hepatocellular carcinoma after pulse power ablation with nanosecond pulsed electric fields". *Technology in Cancer Research and Treatment* 11 (2012): 83-93.

43. Beebe SJ. "Ultrashort pulse induced regulated cell death". In Ultrashort Electric Pulse Effects in Biology and Medicine. Beebe, S. J, Joshi, R, Schoenbach, K.H, Xiao, S.; Springer Singapore (2021): 227-263.

44. Uhl M., *et al*. "Autophagy within the antigen donor cell facilitates efficient antigen cross-priming of virus-specific CD8+ T cells". *Cell Death and Differentiation* 16 (2009): 991-1005.

45. Kroemer G., *et al*. "Immunogenic cell death in cancer therapy". *Annual Review on Immunology* 31 (2013): 51-72.

46. Galluzzi L., *et al*. "Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018". *Cell Death and Differentiation* 25 (2018): 486-541.

47. Guo ZS., *et al*. "Oncolytic immunotherapy: Dying the right way is a key to eliciting potent antitumor immunity". *Frontiers in Oncology* 4 (2014): 74.

48. Yatim N., *et al*. "RIPK1 and NF-κB signaling in dying cells determines cross-priming of $CD8^+$ T cells". *Science* 350 (2015): 328-333.

49. Kepp O., *et al*. "Consensus guidelines for the detection of immunogenic cell death". *Oncoimmunology* 3 (2014): e955691.

50. Inoue H and Tani K. "Multimodal immunogenic cancer cell death as a consequence of anticancer cytotoxic treatments". *Cell Death and Differentiation* 21 (2014): 39-49.

51. Guo S., *et al*. "Nano-pulse stimulation for the treatment of pancreatic cancer and the changes in immune profile". *Cancers (Basel)* 10 (2018): 217.

52. Rossi A., *et al*. "Mechanisms and immunogenicity of nsPEF-induced cell death in B16F10 melanoma tumors". *Scientific Report* 9 (2019): 431.

Volume 5 Issue 8 August 2021