Nano-Pulse Stimulation (NPS) is a non-thermal, localized application of ultrashort electric pulses in the nanosecond range that can trigger immunogenic cell death in treated tumors. We have demonstrated previously that the application of 2000 pulses 100 ns long and 30 kV/cm in amplitude completely ablates the treated tumor within 3 weeks via apoptosis and initiates an immune response that inhibits secondary tumor growth[1]. Here we demonstrate that the application of 2000 pulses, 100 ns long, and 53 kV/cm in amplitude or 1332 pulses, 300 ns long, and 30 kV/cm in amplitude eliminates primary cutaneous melanoma tumors and affords adaptive immune protection against a subsequent tail vein injection with free circulating melanoma cells as evidenced by significantly reduced pulmonary tumor burden.

**Methods**

**Experiment 1:** 14 female B6/J albino mice were given a single intradermal injection of 500,000 B16-GFP cells in 15µL HBSS. Upon reaching 5mm in its largest dimension as visualized by epifluorescence, the tumor in 6 mice was resected surgically, and the tumor in 8 mice was treated with 2000 pulses of 100 ns and 50 kV/cm administered with a prototype pinch applicator at 4 pps. Four weeks after resection or NPS treatment, both the six surgically resected mice and four NPS-treated mice were injected with 200,000 B16-GFP cells into the lateral tail vein. 4 NPS treated mice were not challenged as negative controls. Lung metastases were counted 3 weeks later by epifluorescence imaging.

**Experiment 2:** 19 female B6/J albino mice were given a single intradermal B16-GFP melanoma tumor as above. 8 mice had their tumors resected surgically, and the other 7 mice had their tumors treated with 1332 pulses of 300 ns and 30 kV/cm administered with a second generation pinch applicator at 6 pps. Three weeks after resection or NPS treatment, both groups of mice were injected with 100,000 B16-GFP cells into the tail vein of each mouse as above. 4 NPS treated mice were left unchallenged. Lung metastases were counted 19 days later by epifluorescence imaging.

**NPS Treatment of GFP-Melanoma**

**Results**

**Experiment 1:** After intravenous injection with 200,000 B16-GFP melanoma cells, mice with surgical resection of the primary tumor averaged 17 lung metastases/mouse. Mice with NPS ablation of the primary tumor averaged 3.3 lung metastases/mouse. Mice with NPS ablation of the primary tumor and no challenge exhibited no lung metastases.

**Experiment 2:** After intravenous injection with 100,000 B16-GFP melanoma cells, mice with surgical resection of the primary tumor averaged 19 lung metastases/mouse. Mice with NPS ablation of the primary tumor averaged 10 lung metastases/mouse. Mice with NPS ablation of the primary tumor and no challenge exhibited no lung metastases.

**Conclusion**

NPS treatment of tumor cells results in Immunogenic cell death (see poster #345). This results in the stimulation of the mouse’s immune system to generate anti-tumor immunity to a subsequent challenge with intravenous B16-GFP cells. Therefore, NPS treatment of melanoma tumors not only eliminates the tumor via apoptosis, but also converts the tumor into a vaccine. The subsequent immune response then reduces the rate of metastasis.

**References**