**Oncolytic virotherapy and electroporation: synergistic superheroes for pancreatic cancer?**

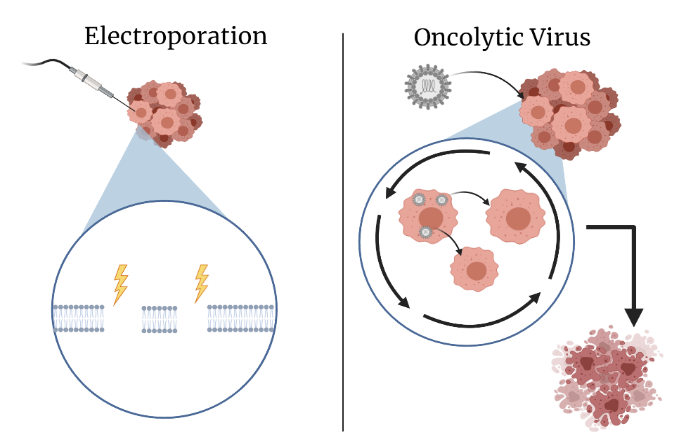
Alex X. Arreola   
Microbiology & Cell and Molecular Biology  
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

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Oncolytic-virotherapy has begun to act as a promising treatment for particular cancers. However, many obstacles stand between it and a cancer-wide cure. Research is underway to investigate means of increasing the effectiveness of this strategy. In fact, a recent experimental combination therapy has shown much promise in the treatment of pancreatic cancer both in vivo and in vitro. In a pre-clinical investigation, it was determined that when the novel oncolytic Alphavirus M1is combined with electroporation therapy, the therapy’s efficacy exceeds the sum of the contribution made by each treatment alone—presenting a synergy. This discovery suggests a hopeful future treatment for a disease that is increasing in mortality. However, further pre-clinical testing is necessary before the therapy can be considered for human trials**.**

**Introduction**

An oncolytic virus (OV) is a promising tool in the battle against cancer. It has two primary avenues of attack on cancer cells. First, it specifically infects and lyses cancer cells. Second, upon lysis, they cause the release of antigens which stimulates the body to unleash a preferential attack on cells that hold these antigens—i.e. the other cancer cells. The first observation that a viral infection could have some sort of anti-cancer effect can date back to the beginning of the 20th Century. In 1904 Professor of Medicine at the University of Michigan, George Dock, observed that Leukemia patients who contracted influenza would sometimes experience periods of remission during their pathogenic infection[1](#Citation1). Despite this, limitations in technology and little success in the field at the time ultimately produced slow progress for the remainder of the century. In the last 20 years, however, there has been a resurgence of investigations into the ability of oncolytic viruses to treat cancers. In 2005, researchers at Shanghai Sunway Biotech created the first genetically modified OV that received government approval as a cancer treatment[2](#Citation2). Since this landmark, many investigations have followed in the pursuit of other effective OVs. In 2015, the United States Food and Drug Administration (FDA) approved the first OV to treat patients with melanoma[3](#Citation3). A major hurdle in the advancement of Oncolytic Virothearapy (OVT) is its specificity and the limited capability of the virus to penetrate solid tumors of particular cancers, such as pancreatic cancer[4](#Citation4). This has spurred multiple avenues of exploration to increase the efficacy OVT. One such route is that of synergies between OVs and other cancer therapeutics. A potential side-kick to OVT is a new tool called NanoKnife which is capable of producing both reversible electroporation (RE) and irreversible electroporation (IRE). This device uses high-voltage, low energy DC current pulses to induce cell death[5](#Citation5). The electrical current imposed on the cells creates small pores on the surface, allowing drugs and other particles to enter that are not usually able to.

***Figure A[7](#Citation7)***

*Effects of electroporation and an Oncolytic Virus on cancer cell*

**Recent Progress**

A paper recently published by a Chinese research team[6](#Citation6) led by Shuxin Sun explored the effects of combining NanoKnife, an FDA approved electroporation machine, and the novel Alphavirus *M1* OV. It was found that NanoKnife indeed enhances the infection of the M1 Virus. Astonishingly, it was observed that their efficacy increases synergistically when administered together and a stronger onco-immune response was elicited. These findings allude to a hopeful future in the realm of pancreatic cancer research—an area in much need of promising findings.

**Discussion**

To reach these conclusions the researchers employed a multifaceted approach. First, they established the capabilities of Nanoknife and analyzed its effects when combined with OV therapy. They then explored the mechanisms of such effects, and observed the generated onco-immune response.

***D.1. Achieving electroporation***

First, Sun’s team explored the variable effects of electroporation induced by NanoKnife. In each trial, they exposed the cells to increasing electrical field intensities. They started with 500 V/cm worked up to 1500 V/cm increasing the intensity by 250 in each subsequent trial V/cm. From the collected data, it was concluded that when electrical field exceeded 1000 V/cm, IRE began to be achieved and as the field approached 1500 V/cm IRE was achieved more frequently—exceeding IRE in over 90% of cells. An important note for further discussion, it was also observed that the pores among the membrane remained opened for approximately 5-10 minutes after treatment in the cells that achieved reversible electroporation (RE). Establishing these parameters for the capabilities of the NanoKnife machine was an essential foundation for subsequent exploration into the possible combination therapy.

***D.2. Synergistic elimination of pancreatic cancer cells***

By understanding that Nanoknife creates pores in the membrane of cells, one can see its potential to aid OVs in the penetration of tumors. Less apparent, however, was the synergistic effect in efficacy that occurred when these two therapies were combined. To begin testing the effects of the combined therapy, the researchers needed to understand the sums of the contributions made by each therapy. First, they examined how efficient the novel M1 OV and NanoKnife alone were in the elimination of pancreatic cancer cells. Once this had been establish, they could explore the potential of the two combined. By calculating the combination index—a mathematical formula used to analyze the contributions of each therapeutic in the effectiveness of the therapy as a whole—based on the data obtained in their cell viability assay it was suggested that the two treatments worked in a synergistic fashion. With these promising findings in vitro, Sun’s team progressed to a mouse model. Here, they sought to recreate their findings in vivo. To do so, the researchers implanted pancreatic cancer cells into immunocompetent mice and waited for the establishment of tumors. Once this was achieved they tested each treatment again monothearpeutically and in combination. Much like the in vitro model, the combination therapy appeared to be much more effective than either of the therapies alone. The fact that data collected both in vivo and in vitro provides a promising suggestion that this combination therapy may have clinical significance.

***D.3. Increased Viral Infection***

With the astonishing results from the combination therapy, the question of “how?” arose. Though the assumption could be made that the effects of electroporation would make it easier for M1 Viral particles to enter the pancreatic cancer cells, the researchers sought to confirm this notion. Here, they examined the viral load in pancreatic cancer cells after a series of NanoKnife treatments. It was concluded that when exposed to the electroporation treatments, there was an elevated amount of viral particles inside the pancreatic cancer cells thus confirming the idea that NanoKnife increased M1 virus infection. To explore in vitro implications, they again transplanted pancreatic cancer cells into immunocompetent mice and established tumors. Based on earlier findings during the investigation of the Nano Knife’s variability, it was determined that RE is unable to persist for longer than 10 minutes. With this considered, they developed two strategies to further solidify the notion that electroporation increases viral infection. First, they tested the effects when the M1 OV was administered 10 minutes after the Nanoknife treatment. After this the tested the effects of administering the Nanoknife treatment immediately after the administration of the M1 virus. The results reflected the expectation. The tumors that resulted from the sequential trial were indeed smaller than those of the control, but they were not nearly as small as those from the simultaneous treatment.

***D.4. Stimulating an immune response***

Both of these treatments alone are known to have promising immune-oncological effects. With this in mind, the investigators decided to examine the antitumor responses generated by these therapies. By analyzing the pancreatic cancer tumors of the mice, it was observed that there was an increase of CD45 leukocytes in the groups of immunocompetent mice that received both the NanoKnife monothearapy as well as the combination therapy. Additionally, it was found that the proportion of T cells in leukocytes were higher in the combination therapy than in either therapy alone. Suns’ team also confirmed through immunohistochemical staining that Cd4+ and CD8+ T cell infiltration increased within the Tumor Micro Environment. Not only was it observed that there was an elevated level of these T cells present after administration of these therapies, but astonishingly, the T cells present during the combination therapy were far more lethal against pancreatic cancer cells

***D.5. Looking Forward***

The findings of this study, undoubtedly shine a hopeful light of into the realm of future therapeutics. But as with many other experimental therapeutics, much preclinical testing remains. Large scale efficacy trials and investigations into the long term survival of specimens must be pursued before any of these finding can be advanced to clinical trials. Even then, it is unknown if there will be any adverse implications in the human trials that limit the progression of this therapy further. Regardless of barriers to come, the synergistic effects of NanoKnife and M1 OVT provide a hopeful outlook for the future treatment of pancreatic cancer.

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7. Figure made on BioRender.com