**Bridging the gap in immunotherapy efficacy for the treatment of pancreatic cancer—the case for oncolytic virotherapy**

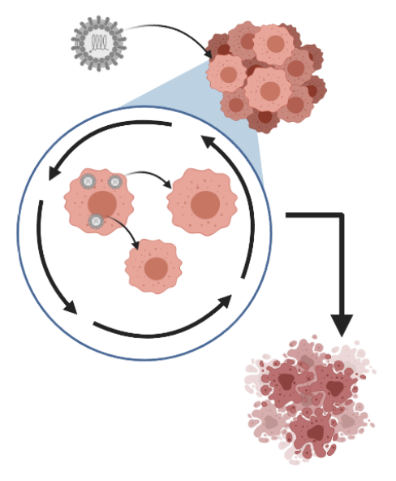
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**In recent times, various immunotherapies have been glorified for their widespread efficacy in treating cancer. However, a deeper look into the effectiveness of such therapies in treating all forms of cancer reveals disparities. One such case is that of pancreatic cancer. Until recently, many roadblocks stood in the way of the immunotherapeutic efficacy for pancreatic cancer. The last two decades have witnessed the development of unique immunotherapy to overcome these blockades—Oncolytic Virotherapy. Though viewed optimistically in concept, further investigations revealed that many barriers bared even this approach from being effective in treating pancreatic cancer. However, within the past year, two key publications suggest that this may no longer be the case. From synergies to macrophage reprogramming, this recently obtained evidence gleams hopeful light into an area of cancer research in much need of advancement.**

**Introduction**

A decreasing trend in death rates can be observed for many cancers, yet pancreatic cancer (PC) is increasing in mortality1. Tumors of the pancreas are often classified by their aggressive growth and resistance to current clinical therapies. A promising new approach in the field of cancer treatment is immunotherapy. One such immunotherapeutic strategy that has gained much attention for its efficacy, is that of immune checkpoint inhibitors. However, PC has been observed to be unresponsive to this strategy2. Thus, a critical need for novel therapies to treat this disease is of great importance. A unique approach that has seen a resurgence of attention within the past decade is oncolytic virotherapy (OVT). In this strategy, a genetically engineered oncolytic virus (OV) specifically infects and replicates inside PC cells. Upon replication, the viral particles will lyse the cancerous cells and continue to infect other cancer cells12. An illustration of this process can be seen in *Figure I.1.* The benefits of this therapy do not end with this amplification of PC-specific killers, however. Once the cells are lysed, antigens are released. This initiates the patient’s own immune system to attack the PC cells that hold these antigens. However, viruses having some sort of anti-cancer effect is no new notion. At the turn of the 20th century, a physician-scientist by the name of George Dock observed a stage of remission in a select number of Leukemia patients who contracted influenza3. Though this observation was made in 1904, very little progress was made until the post-genomic era. In fact, the very first OVT that received a government approval wasn’t until 20054. The approval of the first OVT by the United States Food and Drug Administration (FDA) did not come for another 10 years following this. As with other immunotherapies, OVT was observed to not be as effective against PC as it was with other cancers. This has been partially to blame for the virus’s limited ability to penetrate solid tumors as well as the inaccessibility of the PC tumor. Despite this, recent research suggests that the hurdles reducing OVT efficacy may be able to be overcome.

*Figure I.1 11*

*Lysis of cancer cells via oncolytic virus*

**Recent Progress**

Within the past year, major advances in OVT for the treatment of PC have been made. Two recent publications have presented avenues around the roadblocks mentioned previously. In their publication titled *Combining NanoKnife with M1 oncolytic virus enhances anticancer activity in pancreatic cancer,* researchers led by Shuxin Sun demonstrated enhanced viral infection and anti-cancer activity of an OV when used in combination with electroporation both in vitro and in vivo2. In addition to this, the answer to the inaccessible nature of the PC tumor is suggested in the publication titled *A systemically deliverable Vaccinia virus with increased capacity for intertumoral and intratumoral spread effectively treats pancreatic cancer6.* In this study, Giulia Marelli and their team demonstrated effective use of a systemically delivered OV in Syrian and murine hamster models.

**Discussion**

*D.1 Electroporation of PC Cells*

As stated previously, progress in OVT for PC treatment has been hindered by the virus’s inability to penetrate solid tumors5. In their recent study, Shuxin Sun’s team attempted to overcome this barrier by employing a technique known as electroporation. Electroporation involves applying an electrical field to cells to increase their permeability13. In their study, they utilized the FDA-approved NanoKnife machine to carry out this process. NanoKnife is a recently developed machine that uses high-voltage, low-energy DC current to increase the permeability of the cells. In Sun’s study, the efficacy of the NanoKnife machine was established in a series of trials. The researchers initially exposed the cells to an electrical field of 500V/cm and continued to test in increasing intervals of 250 V/cm until their final trial of 1500 v/cm. From their data, it was observed that when the electrical field was below 1000 V/cm, reversible electroporation of the PC cells persisted for approximately 5-10 minutes. More impressive, however, were the effects observed on the intensities that exceeded 1000 V/cm. Here, it was seen that Irreversible electroporation was achieved. In their final trial of 1500 V/cm, irreversible electroporation was achieved in over 90% of the treated PC cells. Increasing the permeability of the PC cells will allow larger molecules to have access to the intracellular space that was once inaccessible. Thus, employing this strategy in combination with OVT could provide a hopeful option as a novel therapy.

*D.2 Synergistic elimination of PC cells using NanoKnife-OV combination therapy*

The notion that electroporation could enhance the efficacy of OVT is not radical when you consider the complementarity nature of the two therapies. However, to what extent electroporation will enhance OVT is not as easily seen. To test this avenue, Sun’s team explored the effects of NanoKnife monotherapy, OV monotherapy, and NanoKnife-OV Combination therapy*.* Enhanced elimination of the PC cells was clear after the combination therapy was employed. To quantify the extent of the enhancement, they calculated the combination index of the approach. The combination index is a mathematical equation used to test the contributions made by each therapy in the overall effectiveness of the combined therapy. Their computations presented a combination index of less than one, which suggests a synergy exists in the combination therapy. With this astonishing finding, Sun’s team decided to perform similar testing in vivo. Using a mouse model, they tested the effects of the therapies on tumors that resulted from subcutaneously implanted PC cells. Using the same parameters as before, results similar to that of the in vitro study were obtained. From the observations made through this study, the enhancement of OVT when combined with electroporation techniques is clear. The fact that similar results were observed both in vitro and in vivo further suggests the potential for this approach to enhance OVT’s efficacy in the clinical setting.

*D.3 Prospective OV for PC*

As discussed previously, gaining access to tumors of the pancreas is an incredible endeavor. Procedures for pancreatic tumor resection, such as the pancreaticoduodenectomy (Whipple Procedure) are labor demanding, pose serious risks, and involves weeks of recovery7. Thus, administering additional therapies during these procedures is less than ideal. This poses a major obstacle for OVT, as most OVs are limited to intratumoral injection6 and only about 15-20% of pancreatic cancer patients are candidates for surgery8. However, a hopeful contestant has recently emerged. A Vaccinia Virus (VV) engineered by a group of researchers led by Giulia Marelli displays a possible route around this obstacle. Their research indicates that the VV is superior to other OVs in several ways. Some of these characteristics include its lack of requirement for a specific surface receptor, its ability to replicate in hypoxic environments, its induction of immunogenic cells, as well as its ability to induce vascular collapse within the Tumor Micro Environment. Most notably, however, is their finding that the VV can effectively reach tumors through intravenous delivery. Marrelli’s team decided to develop the virus even further to improve its efficacy. They re-engineered a previously studied VV to improve its viral production and spread within tumors. Here, they incorporated an additional copy of the signal peptide, stalk, transmembrane, and cytoplasmic tail. With these changes, they were able to achieve a nearly 6 fold increase of the viral DNA in the tumors of the in vivo model. The findings of this study further support the notion that the efficacy of OVs can be dramatically improved by rationally designed engineering approaches. This further suggests the potential for developing OVT into an effective clinical approach for many PC patients.

*D.4 Enhancing the onco-immune response*

Both of these recent publications highlight key advancements in OVT’s primary avenue of treatment. As mentioned previously, however, OVs have a secondary method of killing cancer cells. Also highlighted in these works are the observations made on the onco-immune response triggered by the OVs. It is known that cancer cells are able to develop mechanisms that mimic peripheral immune tolerance to avoid attack by one’s immune system9. It is also known that OVs are able to counteract these measures by evoking immune activation within the Tumor microenviornment6 (TME). In the study led by Shuxin Sun, an elevated amount of CD45 leukocytes was observed in the TME of the mice that received both monotherapies. More notably, the proportion of T cells and leukocytes were even higher when the OV-NanoKnife combination therapy was administered. The researchers also found that Cd4+ and CD8+ T cell infiltration increased within the TME. These findings are particularly beneficial in suggesting that are hopes to increase viral infection of the solid tumors observed in PC.

In Marrelli’s study, elevated populations of immune cells were also observed. In particular, an increase in CD8+ T cells were seen under various conditions. More notably, in this study, it was seen that their newly engineered VV had the capability of reeducating macrophages to an M1 phenotype. This is a key advancement in OVT as the M1 Macrophage is known to be a strong killer of cancer cells10. The sum of the findings in both studies promotes a positive outlook for immunotherapies in treating PC.

*D.5 The future of OVT*

Though both studies suggest promising advancements of OVT in both in vitro and in vivo models, much larger scale animal studies must be conducted to test to efficacy and safety of these measures before they can even be considered for Clinical trials. Once large-scale evidence is obtained from such studies, many additional OVs will likely be considered for clinical development. This process will likely unfold within the next decade, but in the meantime, researchers will continue to study ways to optimize the efficacy of various OVs. Regardless of the timeline, one thing is certain—the recent improvements of OVT suggest that an effective immunotherapy for treating pancreatic cancer is now closer than ever.

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